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edited by Frans Verdoorn

Volume VI

THE CYTOPLASM *of* THE PLANT CELL

ALEXANDRE GUILLIERMOND *was born in 1876. He obtained his Ph.D. degree at the Sorbonne in 1902 and worked for many years at the University of Lyons. He came to Paris about 1921; at that time he was already famous for his work on the development, sexuality, taxonomy and phylogeny of the Endomycetes and Saccharomycetes; the higher Ascomycetes; the cytology of the Cyanophyceae and Bacteria; starch formation; and especially on account of his research and theories on the chondriome and vacuome. He was appointed Chairman of the Department of Botany at the Sorbonne in 1935, and elected a* Membre de l'Institut *in the same year. Most of his later papers and those of his students (among whom* EICHHORN, PLANTEFOL, MANGENOT *and* GAUTHERET *should be mentioned) have been published in his own journal, the* Revue de Cytologie et de Cytophysiologie Végétales. *He married* HÉLÈNE POPOVICI, *at one time his assistant, the daughter of Professor* POPOVICI *of Iaşi in Roumania. His principal publications are being listed on pages 229/232 of this volume;* cf. *also "Titres et Travaux Scientifiques de* M. A. GUILLIERMOND" *(Lyon / Rey, 1921) and Chron. Bot. II:128* (1936).*

THE CYTOPLASM OF THE PLANT CELL

BY

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AUTHORIZED TRANSLATION FROM THE UNPUBLISHED FRENCH MANUSCRIPT

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FOREWORD

One cannot read this book by Professor GUILLIERMOND without being profoundly impressed with the thoroughness with which it has been written. No one has yet presented, nor is any one for a long time likely to present, so complete and authoritative an account of the mitochondria story. Most of us have been rather bewildered by the prolific, detailed, and contradictory literature on mitochondria, chondriosomes, chondrioconts, chloroplasts, amyloplasts, plastids, etc. It is, therefore, a satisfaction to have it all assembled in a relatively condensed form.

I remember the day, it was a meeting of the Royal Microscopical Society in London in 1921, when a number of cytologists admitted that they were at last convinced that mitochondria are not artifacts. GUILLIERMOND handles the subject of artifacts very convincingly. He is forced to because much of his work has been on fixed material and much of it has been subjected to the usual cry of "artifact".

GUILLIERMOND uses one bit of evidence in support of the reality of certain cell structures which I should like to apply to another part of the cell, the existence of which has also been much questioned, namely, the spindle fibers. Proof of the reality of vesicles is to be had, says GUILLIERMOND, in the fact that they always appear at the same stage of development of the cell whatever the fixative employed. This is likewise true of spindle fibers.

SHIMAMURA has recently offered evidence of the existence of spindle fibers by showing that if a cell in midmitosis is centrifuged and then fixed, the spindle with fibers is found to be centrifugally distorted. Centrifuging cannot distort an artifact before it has been formed by fixation.

Though supporting the reality of mitochondria, Professor GUILLIERMOND in no way enthuses unduly over their possible significance in the life of the cell, as have some other workers. He regards as untenable the theory that chondriosomes are symbiotic organisms. He also discards the idea that chondriosomes are the means by which every synthesis in the cell takes place. In rejecting the theory that chondriosomes are distinct organisms, a concept based on their superficial resemblance to bacteria and the fact that both are stained by the same dyes, GUILLIERMOND shows a breadth of mind characteristic only of the true scholar. He says, that though the theory is untrue, it gave rise to much good research. He concludes the section on the function of mitochondria by the

courageous and laconic statement that "Nothing is positively known about the rôle of the chondriosomes".

Controversial matters GUILLIERMOND handles with fairness and dignity. Each problem is dealt with unemotionally. He refers to SCHLEIDEN "as the promulgator of the cell theory"; to ROBERT HOOKE as the first to recognize the cellular organization of living things, but the significance of this structure was not understood for a long time thereafter.

I anticipated a rather orthodox cytological handling of the subject by GUILLIERMOND, and so was pleased to find him as awake to the contributions of the newer cytology as to those of the old. He takes DE JONG's concept of cytoplasm as a coacervate and applies it to chondriosomes; vesiculation indicates that they too are coacervates.

GUILLIERMOND deals with the question of the physical nature of the tonoplast. I should like to restate it somewhat differently, and what I say of it is also true of the outer surface layer of protoplasm. All protoplasmic surfaces are probably coated with fats or other substances which are immiscible with water, but this does not mean that cell membranes are made of any substance other than protoplasm. Cell membranes are immiscible with water because protoplasm is immiscible with water. The immiscibility of protoplasm in water is not primarily due to an oily surface. Structural continuity is responsible. Protoplasm takes up water just as does a sponge, silica gel, or gelatine. Living matter is not a solution of salts, sugars, and proteins. Protoplasm holds together. It could not be a living system if it did not do so. Such misunderstandings have arisen because protoplasm shows certain properties of liquids, such as rounding up and flowing. To certain students this can only mean that protoplasm is a liquid, and if it is a liquid it must be a solution. The colloidal viewpoint clarifies all this. Protoplasm does flow and therefore it is a liquid, but it is elastic, possesses tensile strength and contractility, and imbibes, that is to say, soaks up water. These are the properties of solids, their presence in protoplasm indicates structural continuity.

Protoplasmic membranes, whether the inner tonoplast or the outer cell membrane, are of living matter, capable of the same physical and chemical changes as is the protoplasm which they bound. The cell membrane is not an inert layer of oil; it is a dynamic living system.

When amoebae and slime molds move forward, the advancing surface is in a constant state of change. As the surface increases in area, material is added from the inner protoplasm, and as it decreases in area part of its substance is returned to the inner protoplasm. In short, the membrane is protoplasm. Convincing evidence of this is an interesting observation which led one of my students to flatly deny the existence of the tonoplast when it should have caused him to recognize that protoplasmic membranes are alive; that they are dynamic not static systems. He had observed a particle within the vacuole of a plant cell moving at the same rate and in the same direction as the streaming protoplasm. Close observation revealed that not only the mass of protoplasm but its

surface, that in direct contact with the vacuolar sap, was also in an active state of flow. It was this streaming interfacial protoplasm which caused movement of the vacuolar sap and the particle within it. There was therefore no quiet layer between protoplasm and sap, which meant to my student that there was no tonoplast. He failed to appreciate that the tonoplast and cell membranes in general are not inert skins but a surface layer of living protoplasm. The tonoplast *is* protoplasm; in that sense is it formed of a substance immiscible with water. It is, as HUGO DE VRIES said, "a membrane differentiated and living".

Much of the misunderstanding in regard to protoplasm arises from a failure to realize that protoplasm possesses both liquid and solid properties. Its liquid character is real but superficial. Its solid qualities are basic. That protoplasm is liquid is evident from the fact that it flows, but it is in no way comparable to a solution of salt in water. Viewing protoplasm as a liquid devoid of solid properties was an excusable fault in the classical cytologists, but the modern physiologist is aware, as is GUILLIERMOND, of the true physical nature of living matter and the need of structural continuity. GUILLIERMOND refers to this indirectly by attributing to protoplasm such properties as torsion, elasticity, and immiscibility in water. WARREN LEWIS once expressed the need of structural continuity in protoplasm when he stated that were it not for the glutinous qualities, the tackiness of protoplasm, we should all fall to pieces. In short, protoplasm holds together, and this is as true of fluid protoplasm as of firm protoplasm.

GUILLIERMOND concludes this book with the statement that the future of cytology lies in the union of morphology and physiology. In return for the admission by a morphologist that anatomy without physiology is sterile, let me say to Professor GUILLIERMOND that physiology is meaningless unless supported by structure and function.

The present volume is the first addition, printed in the New World, to the list of books which Dr. FRANS VERDOORN is editing and publishing under the title of "A New Series of Plant Science Books". The book was especially written for the "New Series" by Professor A. GUILLIERMOND; it is the translation of an unpublished French manuscript and not merely an English version of a previous book by GUILLIERMOND.

The translator is Mrs. ATKINSON of Amherst, Massachusetts (Ph.D. University of Wisconsin, sometime C.R.B. Fellow in Botany, University of Louvain). Mrs. ATKINSON has given us far more than a translation of the original manuscript, for there was much interpretation and rearrangement to be done. The translator is to be congratulated on accomplishing a difficult piece of work so well. Valuable help in editing the Ms. was also rendered by Dr. J. DUFRÉNOY of Louisiana State University.

September 1941

WILLIAM SEIFRIZ

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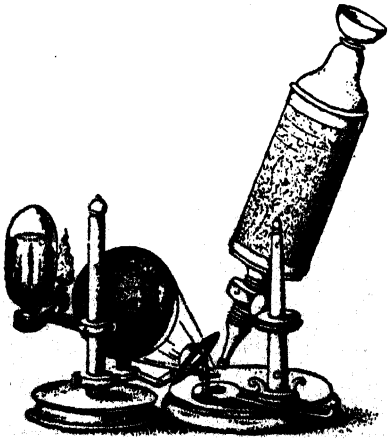


Chapter I

INTRODUCTION

Historical sketch:- It is well known that the cellular organization of living things was first recognized by the English engineer, ROBERT HOOKE (1665), who, toward the end of the seventeenth century, was looking at sections of cork with a view to finding out what applications could be made of the recent discovery of the microscope. He described the tissue as formed of alveoli resembling a honey-comb. These alveoli he called *cells*. For a long time, however, the significance of this structure was not understood. The French botanist, BRISSEAU DE MIRBEL (1833) thought that cellular tissue was composed of vacuoles hollowed out of a homogeneous substance, which corresponded to living matter. It was MOLDENHAWER (1812) who, for the first time, demonstrated the individuality of cells. Having succeeded in separating the elements of tissues by maceration, he proved that cells have a wall of their own and cannot, therefore, arise as cavities in a homogeneous substance. Later DUTROCHET (1824), TURPIN (1827) and MEYEN (1830) considered cells as morphological entities but their attention had been centered rather on the walls than on the contents of the cells. In 1830 MEYEN had discovered chlorophyll grains, starch grains and crystals within the cavity of the cell. In 1831 the English botanist, ROBERT BROWN, who gave his name to Brownian movements, discovered the nucleus in the epidermal cells of orchids. Shortly after (1838), SCHLEIDEN, the promulgator of the cell theory, attributed predominating importance to the nucleus to which he gave the name *Cytoblast* and which he considered as the generator of the cell. According to SCHLEIDEN, a cell is formed as follows: in a matrix, the cytoblastema, there appears the cytoblast on whose surface a membrane then becomes differentiated which lifts itself up like a watch crystal, grows, and bursts away from the cytoblast, leaving an empty space into which the matrix penetrates by filtration.

DUJARDIN (1835), in the cells of Infusoria, first accurately described living matter, to which he gave the name *sarcode*. NÄGELI (1866) perceived in addition that plant cells are occupied by nitrogenous matter and VON MOHL at the same time described it under the name of *Protoplasma* and attributed to it a primary importance. According to this observer, the plant cell, made up of the protoplasm, contains a nitrogenous primordial utricle, lining its wall on the inside and enclosing the nucleus. This primordial utricle is the seat of special movements already seen by B. CORTI (1772) and TREVIRANUS (1807). The rest of the cell is occupied by cell sap.

A little later, COHN (1850), THURET (1850) and PRINGSHEIM (1854) perceived that the zoospores of the algae lack a wall and are made up exclusively of protoplasm. MAX SCHULTZE and DE BARY (1859) finally established the fact, once for all, that the protoplasm of plants is the essential substance of cells and corresponds to the sarcode of DUJARDIN. LEYDIG (1857) defined the cell as "a mass of protoplasm furnished with a nucleus". MAX SCHULTZE (1861) defined it as "a mass or lump of protoplasm endowed with vital properties".

While these conceptions were being established, the works of VON MOHL, MEYEN and NÄGELI were proving the inexactitude of SCHLEIDEN's theory of cell origin and showing that cells multiply by division. Thus the word cell (French *cellule*, from the Latin *cellula*, little room) came to mean the contents of the cellular cavity, a significance quite different from that given it by HOOKE, who observed only the walls.

From then on, the conception of the cell was enlarged upon but the knowledge of its structure, making only slow progress, was to remain obscure for a long time. The early cytologists observed only living cells. This presents serious difficulties, for, with the exception of unicellular organisms, observation of living material can be carried out only after tearing or sectioning tissue, operations which risk injuring the cells. Cells examined in a medium not their own — water, for instance — may, during observation, undergo serious alterations. Lastly, observation of living material never allows the study of cell structure to be pushed sufficiently far, because, except for cases which are unusually favorable, the different elements which constitute the cell show too small differences of refractivity for it to be possible to distinguish one from the other with clearness, and this becomes, moreover, well nigh impossible in embryonic tissue in which the cells are very small.

The introduction of the paraffin method on objects previously "fixed" has greatly facilitated the work of cytologists. This method consists in fixing, *i.e.*, coagulating, the cells by means of various chemical reagents, then embedding the tissues in paraffin, cutting them in thin sections with the aid of a microtome and finally staining them. Several stains may then be applied to the sections, which fix this constituent or that, according to its affinity for the stain, and superb preparations may be obtained and made permanent in Canada balsam. But this method, convenient as it is, has, nevertheless, the serious difficulty of reducing cytologists to the study of dead cells only. Fixation, *i.e.*, coagulation, of protoplasm, modifies the structure of the cell and exposes cytologists to serious errors in interpretation. Finally, this method does not allow the physical character or biological properties of the cytoplasm to be studied, although they are very important, and cytology is reduced to pure cellular morphology. The use of the paraffin method has led to the striking discovery of karyokinesis and has rendered very great service in the study of the nucleus and, in par-

ticular, of the chromosomes, whose form in the fixed material is preserved with a minimum of distortion.

On the other hand, as far as the cytoplasm is concerned, the paraffin method has, over an extremely long period, given only mediocre results and cytologists have made the mistake of neglecting too greatly the study of living material. As a matter of fact, the most convincing data on the cytoplasm were for a long time obtained exclusively from living cells: on the one hand, the excellent discoveries of SCHMITZ concerning the chloroplasts of the algae and those of WILHELM SCHIMPER and ARTHUR MAYER in the study of plastids of higher plants, and, on the other hand, the classical experiments of HUGO DE VRIES on the vacuoles and their rôle in the osmotic phenomena of the cell.

It is only since 1910 that knowledge of the cytoplasm has made rapid progress. At that date, the timely appearance of the ultramicroscope enabled A. MAYER and SCHAEFFER to make known some essential data on the colloidal nature of the cytoplasm of animal cells — data which can be applied to plant cells. At about the same time, the introduction of *mitochondrial methods,* new fixation techniques for certain cytoplasmic lipides, led to the discovery of the chondriosomes and made it possible to preserve the plastids, to stain them clearly and to follow them through their whole life history. A little later, the methodical use of vital dyes, which accumulate in the vacuoles of living cells, made it possible to follow the evolution of the vacuoles through all the stages of cellular development. Finally, the invention of the micromanipulator and of motion-picture photography, and the perfecting of methods of observation *in vivo,* have contributed in large part to making known to us the physical properties of the cytoplasm and of its various morphological elements.

Difficulties in the study of cytoplasm. Recommended method:- As a result of work carried on during the last twenty years by means of the mitochondrial technique, or with the aid of the ultramicroscope, or by the use of vital dyes, it has been possible to solve definitely the problem of cytoplasmic structure. The discoveries in this domain are still too recent to be accepted by all cytologists. But if these are still being discussed, it is simply because they have been arrived at by methods very different from those usually employed and because some cytologists are unable to verify them by their own methods. The study of the cytoplasm, a substance which is very easily injured, is infinitely more difficult than the study of the nucleus. It is necessary to use a long and delicate method whose general outline will be indicated here.

This method, which will be called the *analytical method, i.e., analysis of the cell,* consists of the following serial operations: First, this method requires *the use of fixed and stained sections* which are indispensable in bringing out the elements which are considered as entering into the constitution of the cytoplasm. But this method is always insufficient, for once these elements have been

brought out by fixation and staining, their actual presence must be checked by *observation of living material* in such cells as lend themselves to it. This observation of living material permits a proper appreciation of the value of the above methods. As the very handling of living material runs the risk of producing alterations, every precaution to avoid them must always be taken. Fungi may be used which can be observed in their own environment, or organs studied which are sufficiently transparent to permit their cells to be examined without any manipulation. Petri dishes of a special type (used at the International Bureau for the Culture of Fungi at Baarn) can be used if necessary. In the bottom of these is an opening of 3 cm. which can be covered with a slide sealed with asphalt cement (Fig. 88). In this way, seeds can be grown aseptically and their roots during development can be observed with an oil-immersion lens by turning the dish under the microscope. *Vital dyes* must be used to enable us to follow such elements as the vacuoles which are not well preserved by any other means. Moreover, each element whose presence has been recognized by fixation and staining must be described by means of a systematic study of its behavior with most fixatives and stains; this study must be based upon histochemical reactions of the element in question. This method, called *histochemical analysis,* permits us to characterize the element, to distinguish it from others, and to inform ourselves as to its chemical nature. It permits us, besides, to distinguish some elements which are revealed only by certain methods of fixation and staining. Histochemical analysis must be followed by an *histophysical analysis, i.e.,* analysis of the physical state of the element, its viscosity, colloidal state, and so forth, an analysis in which will be employed the micromanipulator, the ultramicroscope, the polarizing microscope, the centrifuge, the plasmolytic method and, if need be, motion-picture photography. This histophysical analysis will supplement the description obtained by histochemical analysis. Then, too, the development of the element throughout the entire life of the cell must be followed in order to know whether it constitutes a permanent element or is only transitory, and in order to obtain an idea as to its significance by observing its behavior. Finally, it will be useful to supplement this method called the *analysis of the cell,* which comprises the various operations just enumerated and which has, itself, all the value of an experimental method, by a further series of experiments designed to clarify the rôle of the element studied. Among these experiments are vivisection, depriving a cell of an individual element by means of the micromanipulator, and a study of nutritional influences on the behavior of the element under consideration. This is the *cytophysiological method* which we will turn to only occasionally here, our aim being chiefly the morphological study of the cytoplasm. *The procedure specified is the only one* by which precise facts may be obtained on the morphological constituents of the cytoplasm: the plastids, chondriosomes and vacuoles.

Chapter II

GENERAL FACTS ON THE STRUCTURE OF THE PLANT CELL, ITS CYTOPLASM AND MORPHOLOGICAL CONSTITUENTS

The cytoplasm and its permanent inclusions:- In agreement with STRASBURGER and HENNEGUY, the term *cytoplasm*[1] is here understood to mean all the living matter in the cell with the exception of the nucleus. By the term *protoplasm* is meant all living matter in the cell, *i.e.*, both cytoplasm and nucleus. The cytoplasm occurs in living cells as a colloidal substance, hyalin and homogeneous, elastic and of a viscosity which is always superior to that of water. This substance holds permanently in suspension a certain number of small elements which resemble bacteria in form and dimensions and are distinguished in living cells by a refractivity slightly higher than that of the cytoplasm. These elements, which are called *chondriosomes*, appear in the form of granules, rods and threads.

In addition to these elements there are, in green plants, the *plastids*, whose form is very variable in the algae and which, in higher plants, appear in green tissue as large globules, filled with chlorophyll, which are derived from small elements very similar to chondriosomes in form and histochemical constitution. The cytoplasm also contains small fluid cavities called *vacuoles*, composed of water, containing crystalloid and colloidal substances. These vacuoles, which are very small and very numerous in young cells, swell and usually run together little by little, to form, in mature cells,

[1] VON MOHL designated under the name of *protoplasm*, the living substance of the cell, *i.e.*, the nucleus and that which in the present volume is called the *cytoplasm*. But STRASBURGER, HENNEGUY, and most of the modern cytologists have reserved the term protoplasm for the cell contents (with the exception of the wall), including the nucleus. Within the protoplasm they distinguish the nucleoplasm or karyoplasm which is the nuclear substance, and the cytoplasm, which is everything else. The term protoplasm, however, is often used as a synonym for that which, in this volume, is termed cytoplasm. HARDY means by cytoplasm all that is not nuclear and he considers the protoplasm to be all living matter within the cytoplasm to the exclusion of the nucleus: the cytoplasm therefore includes the protoplasm and the products of its activity, various inclusions not pertaining to the living substance. BOTTAZZI, on the contrary, incorporated the nucleus into the protoplasm or *bioplasm* which, for him, includes all the living substance of the cell, *i.e.*, the nucleus, the living ground substance, which is not nuclear, and the chondriome. He reserves the term cytoplasm for all the cell contents, *i.e.*, the total protoplasm and all the products elaborated by it. Among these last, BOTTAZZI distinguishes: 1, the *metaplasm* including the products of cellular elaboration which are permanent (cell walls); 2, the *paraplasm*, represented by the reserve substances or waste products, which are only transitory in the cytoplasm (starch grains, fat globules, etc.), as well as soluble substances formed by cellular metabolism (glycogen, inulin) which may be detected by certain chemical reagents. Thus, for BOTTAZZI, protoplasm means all that is living in the cell including the nucleus and the chondriome, whereas the cytoplasm includes the protoplasm and the products of its elaboration (metaplasm and paraplasm).

an enormous single vacuole which occupies the greater part of the cell, forcing the nucleus to the periphery. This vacuole is often traversed by thin cytoplasmic trabeculae which radiate from the nucleus to join the parietal cytoplasm.

Lipide granules are encountered in almost all, if not in all, cells. They are scattered in the cytoplasm in more or less considerable numbers according to the cells and their stage in development. One also finds, moreover, various inclusions in the cytoplasm: reserve or by-products formed during cellular activity (starch grains, crystalline proteins, various crystals, etc.) which are localized either in the cytoplasm itself or in the plastids or in the vacuoles. All these substances, however, are only transitory products formed during cytoplasmic activity.

The paraplasm:- We now turn to a new consideration. It has just been seen that in the cytoplasm there are in suspension some elements which are always present such as plastids, chondriosomes, vacuoles and lipide granules. Among these elements, a distinction must be made between those which can be considered as belonging to the living substance, to the architecture of the cell, and those which simply result from its activity. Among the latter whose chemical composition is more simple, there are some, like the starch grains, which form in the plastids, others, like many crystals, which are localized in the vacuoles, others still, like the crystalline proteins, which are contained in the cytoplasm itself. Now, the cytoplasm is a substance which continues to exist permanently. It presents a chemical composition still not well known but which does not vary appreciably. It is a living substance. The plastids are elements which are never formed *de novo* but, like the nucleus, are transmitted by division from cell to cell. They, therefore, may also be considered as living substance. The chondriosomes seem to be of like nature. Such is not the case for the vacuoles. Although always present in the cells, they appear to arise *de novo* and to disappear only to be replaced by new ones. Furthermore, they enclose substances which, all of them, are products of cytoplasmic activity: reserve products, or waste products, or transitory products of metabolism. They do not, therefore, seem to belong to the living substances of the cell. This is true for the lipide granules which are present in almost all cells, but which vary greatly in quantity depending on the state of development of the cells: there are cells which contain only a few, others in which the granules accumulate in great quantity and fuse to form large globules filling up the cytoplasm. These are also products of metabolism. Coexistent with these visible products, there are others, like glycogen,

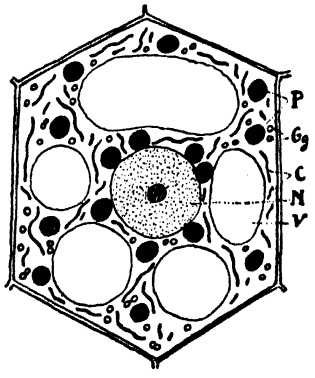


FIG. 1. — Diagram of a plant cell. *C*, chondriosome. *Gg*, lipide granules. *N*, nucleus. *P*, chloroplasts. *V*, vacuole.

which impregnate the cytoplasm and can be detected only by using certain microchemical reagents. All these products arise by cytoplasmic activity and a distinction must therefore be made between them and the cytoplasm by which they were produced. These products are grouped under the term *paraplasm* or *deutoplasm* and are separated from the cytoplasm, which, with the plastids and chondriosomes, constitute living substance. Among the products resulting from the activity of the cytoplasm are some which are of permanent character, such as the cellulose wall. This is a cytoplasmic secretion which persists during the entire life of the cells and can not, it would seem, be considered as belonging to living substance. These permanent formations of the paraplasm or deutoplasm are specified as *metaplasm.* Lastly, the name *protoplast* is used to designate all the contents of the cell except the cell wall, *i.e.*, the protoplasm and paraplasm together.

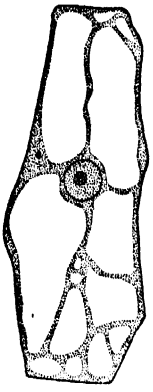


FIG. 2. — Semidiagrammatic representation of a living epidermal cell of *Allium Cepa* showing cytoplasmic trabeculae traversing the vacuole and uniting the parietal cytoplasm with the nucleus.

In the cytoplasm, then, we shall have to consider the cytoplasm itself, the plastids, the chondriosomes and the paraplasm, the most important constituents of the last category being the vacuoles and the lipide granules.

These, and other paraplasmic formations not listed above, will be studied in the succeeding chapters.

Chapter III

THE PHYSICAL PROPERTIES AND GENERAL CHARACTERISTICS OF THE CYTOPLASM

Its appearance in living form:- DUJARDIN, who first studied the cytoplasm in living cells of ciliated Infusoria, has given an absolutely exact description of this substance which he calls *sarcode*, a description which modern observations merely confirm. "This substance", he says, "appears perfectly homogeneous, elastic and contractile, diaphanous and refracting light a little more than water and much less than oil. One can distinguish in it absolutely no trace of organization: neither fibre nor membrane nor an appearance of cellular form".

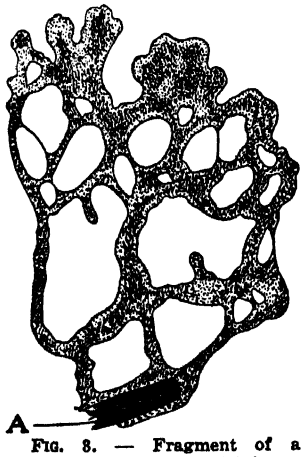


FIG. 8. — Fragment of a plasmodium of *Chondrioderma difforme*. *A*, ingested foreign body. × about 50. (After ZOPF).

There is nothing to add to this description. The cytoplasm appears to modern observers just as it was described by DUJARDIN. In living cells, it appears to be a homogeneous substance, as transparent as glass, viscous, a little more refractive than water and non-miscible with it. As has been already stated, modern research has shown that it does, however, always contain in suspension numerous granules (chondriosomes, lipide granules) and vacuoles, of which more will be said later. Hence the description of DUJARDIN can be applied only to the cytoplasm itself, omitting these elements which it contains.

For the study of the physical properties of the cytoplasm, the plasmodium of the Myxomycetes has been much used. It is seen as a voluminous protoplasmic mass of irregular appearance, lobed in the most fanciful manner and enclosing numerous nuclei. This protoplasmic mass changes shape constantly by virtue of the amoeboid movements which control its displacement. It glides along the surface of its support and if this latter be of decaying wood, it worms its way into the interior of the wood, penetrates it only to come out again further on, then to re-enter it, and so on. The huge dimensions of the plasmodium make it a valuable object for the study of the cytoplasm. The classical experiments successfully performed by PFEFFER on the plasmodium of *Chondrioderma difforme* have shown that in order to alter a small portion of cytoplasm, it is necessary to exert a pressure of 8 mg. per sq. cm. The cytoplasmic strands of this plasmodium break when subjected to a tension of 120-130 mg. per mm. This indicates a rather strong cohesion which, it may be said, can be even stronger in other types of cytoplasm.

Viscosity:- There has been a good deal of discussion concerning the cohesive state of cytoplasm, *i.e.*, its consistency. Most cytologists consider that the cytoplasm more nearly approaches a liquid than a solid state. A few, however, believe it to be of a solid consistency.

The plasmodium of the Myxomycetes is very fluid. A proof of this is in an experiment carried out on *Badhamia utricularis* by the English mycologist, LISTER. LISTER noticed plasmodia of this fungus on the trunk of an old hornbeam growing in his garden. The trunk was covered over with the fruiting bodies of *Corticium puteanum.* The plasmodia of *Badhamia* moved around on the surface occupied by *Corticium,* actually consuming the fruiting bodies and after their passage leaving the bark of the hornbeam as smooth and clean as if no fungus had ever grown there. But, although *Badhamia* assimilated the *Corticium* tissues, its spores, protected by a resistant brown membrane, were not attacked and accumulated within the plasmodium which took on a dark brown color. LISTER collected one of these plasmodia on a glass plate, where it moved about, leaving behind it as evidence of its passage, a fine brown network, formed of the ingested spores. These had been progressively dropped, being poorly retained in the plasmodial cytoplasm. This demonstrates its weak viscosity. The plasmodium, however, still enclosed many spores. LISTER then put in its path a barrier of wet cotton. The plasmodium passed through rapidly, leaving in the cotton all the remaining spores, and emerged showing the yellow tint characteristic of it before it had taken up the fungus *Corticium.* LISTER thus brought about the filtration through cotton of plasmodial protoplasm and in this way succeeded in demonstrating its very fluid consistency.

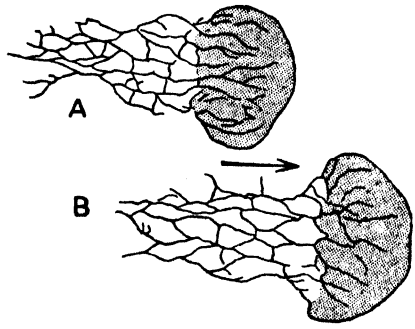


FIG. 4. — Two successive shapes, A, B, taken by the plasmodium of a Myxomycete as it moves in the direction of the arrow.

This somewhat crude evidence may be made more specific by a detailed examination of the plasmodium. It is composed of a network of opaque, anastomosing veins which, in certain species, may attain large dimensions: even a diameter of several millimeters in the case of the principal veins. Toward one border, the network merges with a fan-shaped continuous layer of the same material. This entire body moves at about the rate of 1 cm. per hour and presents constantly changing contours; nevertheless the continuous layer is always in front and may be interpreted as protoplasm flowing slowly over the substratum. The substance composing the plasmodium is, in fact, fluid and motile but shows at its surface the ability to coagulate. A puncture in a vein allows a drop of the internal fluid protoplasm to escape. This drop is immediately coagulated on the surface. The internal protoplasm,

then, under its immobile surface, flows with extreme rapidity. In addition, the different veins and the flowing, continuous layers comprising the plasmodium, show very curious, rhythmic but not synchronous, pulsations. These are rendered quite visible by motion-picture photography. Each vein shows an alternation of systole and diastole; each outer layer flows by jerks over the substratum, becoming alternately thicker and thinner (COMANDON and PINOY, SEIFRIZ).

Under sufficiently high magnification the plasmodium seems to be formed of an homogeneous substance, holding in suspension innumerable granules, in particular, lipide droplets and ingested debris, whose incessant displacement in different directions reveals with great clearness the existence of cytoplasmic currents.

These currents are very irregular. They may be rapid or may even rush along in a vein and, at a given instant, they will immediately slow up, then change direction, accelerate, retard again, return to the original direction and so on. In each vein the same irregularities are observed but without any synchronism whatever. Thus, the movement of the entire mass of the plasmodium in one direction expresses the sum of all these movements in different directions and indicates that in this apparent disorder, the protoplasm flows more in one direction than in the other; namely, in the direction of the advance of the organism.

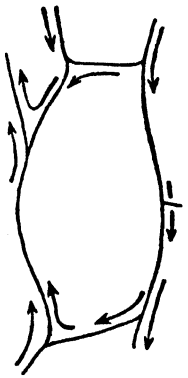


FIG. 5. — Diagram of the different directions taken by protoplasmic currents in a portion of the vein-like reticulum of a plasmodium.

Now these characteristics of protoplasm have nothing unusual about them. Microscopical examinations of the contents of the most varied cells reveal that there, too, protoplasm behaves as a fluid substance. The bodies which it holds suspended in it are in most cases more or less rapidly carried along in its multiple currents. Here, as in the extended body of the plasmodium, these currents run side by side, separated by calm borders. They ramify, anastomose and change constantly. These are the phenomena of *cyclosis* for which the cells of *Elodea canadensis* and the staminate hairs of *Tradescantia* or of Celandine are classic subjects for observation. These phenomena are found again in *Spirogyra* in which they appear with great distinctness and of which more will be said later on.

All these facts indicate, therefore, that cytoplasm flows. This presupposes a mobility of molecules found only in a liquid state, which, in a word, is the essential property of a liquid state. These movements are, however, much less accentuated in some cases. They seem to be nearly absent in certain plant cells, among others in yeasts and various fungi, in which the cytoplasm appears to be much less fluid and shows no displacement of granules. This seems also to be the case in most animal cells.

Other arguments resting especially on observations of plant cells have been brought forward in favor of the fluid state of cyto-

plasm. HOFMEISTER and BERTHOLD showed for the first time that if a section is cut out of a filament of *Vaucheria*, a part of the cytoplasm comes out, taking a spherical form by virtue of the law of surface tension which characterizes liquids (Fig. 7). Since that time, many similar experiments have been performed, notably by STRUGGER on *Chara* cells. And finally, an experiment by KÜHNE on the cytoplasm of the Myxomycetes, can be explained only by a liquid state of the cytoplasm. This worker succeeded in obtaining an artificial muscle by enclosing in elastic tubes, fragments of the plasmodium (intestines of *Hydrophilus piceus*).

All these facts, added to those obtained by microdissection, which will be spoken of further on, permit us to conclude that the cytoplasm possesses, in general, the properties fundamental to liquids: *it flows and has a surface tension which tends to make it take the form of minimum surface, i.e., spherical form.*

Research carried out in these later years with the aid of the microdissector, put into practice by CHAMBERS, has made great progress in the knowledge of the viscosity of the cytoplasm. This method consists in the use of a special instrument, the micromanipulator, or microdissector, provided with glass needles which can be moved mechanically with great precision. This permits the dissection of cells under high magnifications.

The work of SEIFRIZ with the micromanipulator on various plants (Mucoraceae, *Fucus*, pollen tubes, plasmodia of Myxomycetes) also demonstrates that the cytoplasm presents a consistency which is very variable: now very fluid and almost like water, now almost solid, even to the state of a gel with a consistency of bread dough or vaseline, in which the passage of the needle leaves a gaping hole.

FIG. 6. — Cyclosis in a staminate hair of *Chelidonium*. The arrows indicate the direction of the currents in the various cytoplasmic meshes. *n*, nucleus. (After VAN TIEGHEM and COSTANTIN).

The cytoplasm is very fluid in the plasmodium of the Myxomycetes as long as the latter is in an active state. If the point of a needle of the micromanipulator is broken off in the plasmodium, the cytoplasm is rapidly aspirated (*cf.* p. 37). Its fluidity is, however, always greater than that of water, intermediate between that of water and that of oil of paraffin, but from the moment that the plasmodium ceases growth, *i.e.*, in the stages which precede sporulation, the consistency of the cytoplasm increases: it equals that of oil of paraffin, then that of glycerin and, finally, that of bread dough. In *Rhizopus nigricans*, the cytoplasm is also liquid in the young portions of the hypha. It is, nevertheless, less fluid than that of the Myxomycetes and presents approximately the consistency of oil of vaseline. On the contrary, in the older portions of the hypha of *Rhizopus*, the cytoplasm grows thick. Its viscosity becomes that

of glycerin and then that of bread dough and may even attain and exceed that of vaseline.

In the egg of *Fucus* the cytoplasm becomes more and more viscous as the egg matures and its exchanges diminish. Immediately after fertilization it again becomes liquid and remains in this state in the embryo. Areas seem to exist in the egg, therefore, where fluidity is more accentuated,—sort of centers of activity where different chemical exchanges are produced.

It appears from all these observations then, that the viscosity of cytoplasm is always superior to that of water and in numerous cases as great as that of blood. Yet in certain cells, especially in old organs, the viscosity can be much higher. Moreover in dehydrated organs, seeds, for instance, the cytoplasm can be more or less solid.

Many attempts have been made to measure directly the viscosity of the cytoplasm inside the cells. The easiest method to employ is that which makes use of the law for falling spheres established by the physicist STOKES. The cytoplasm of certain cells encloses starch grains which are more dense than the cytoplasm and which have a tendency, because of their weight, to fall through the cytoplasm to the lowest point in the cell. By using an horizontal microscope, the time required for the grains to fall is determined and, by applying the physicist's formulae, the viscosity of the cytoplasm is calculated.

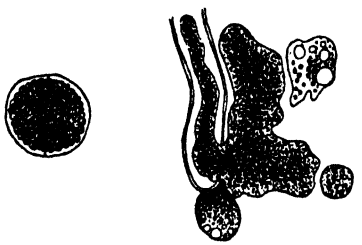


FIG. 7. — Ruptured siphon of *Vaucheria* showing tendency of lobed extruded contents to break up into globular drops. At left, one droplet greatly magnified. (After VAN TIEGHEM and COSTANTIN).

By this process of determining the speed with which starch grains and certain crystals fall through the cytoplasm, WEBER was able to state that the viscosity of the cytoplasm varies with the cell under consideration. It seems to increase with the age of the cells. Certain influences (increase in temperature, the action of certain chemical substances, such as narcotics) can also cause variations.

HEILBRONN has employed the following method: an iron needle, very fine but too heavy to be carried about in the cytoplasmic currents, is introduced into a plasmodium. The needle being located under the microscope, an electro-magnet in which flows a current of increasing intensity, is brought near the preparation. When the needle in the field of the microscope begins to orient itself in the direction of the lines of force, the experiment is stopped. The intensity of the current necessary to produce a visible deviation of the needle is divided by the intensity producing the same effect on the same needle plunged into pure water. With the viscosity of water thus taken as a standard, the quotient measures the viscosity of plasmodial protoplasm.

The centrifuge has also been used on living cells. This brings about a displacement of heavy inclusions, such as starch grains, or of light inclusions, such as lipide granules, with a speed and facility varying with the consistency of the cytoplasm. This method is much less precise since the exact densities of the granules and cytoplasm are not exactly known.

These various methods have shown that the most fluid cytoplasm has a viscosity which is only 3-5 times that of water and that the most dense cytoplasm (that of animal cells) reaches nearly 10,000 times the viscosity of water. Thus from the results obtained by means of centrifuging, microdissection and other techniques, an essential and very general conclusion may be drawn: the cytoplasm of plants does not present at all times the same viscosity, for this varies essentially with the physiological state of the organ under consideration.

Rigidity:- The cytoplasm possesses, at the same time, a property which, as will be seen later, is tied up with its physical state and which is characteristic of cells, namely, a certain rigidity which gives to it an elasticity of torsion. Rigidity expresses the physical ties between the particles of the system in question, ties which are lacking in true liquids. This rigidity can be brought out by micromanipulation. Thus, in displacing cellular inclusions within the cell, it was observed that sometimes these return to their places when pressure is released, the rigid surroundings acting as a spring, and sometimes the inclusions are displaced as from a liquid without rigidity. The stability or instability of form of these inclusions after being deformed, for instance being drawn out between two needles, also teaches us something of their rigidity and their variations. In this way, SCARTH, for example, demonstrated the elasticity of the cytoplasm of *Spirogyra*. The nucleus of one cell of this alga, when pushed by a microneedle from one side of the cell to the other and then left alone, was observed to return of itself to its original position. Like viscosity, rigidity seems to be variable in the cell. Microneedles sometimes penetrate very easily into a fluid cytoplasm without reaction, and sometimes with difficulty into a thick gell. There is no method for measuring the rigidity of the cytoplasm.

Density:- By a micropycnometric method, LEONTJEW succeeded in measuring the density of the plasmodium of *Fuligo septica* and has shown it to be, on the average, 1.040 for individuals collected in dry weather while it does not surpass 1.016 for those collected in wet weather, but eleven hours after sporulation it rises to 1.065.

Ectoplasmic layer:- The cytoplasm is not miscible with its external surroundings but remains always very sharply separated from them. In cells with no skeletal walls, the cytoplasm is surrounded by an external zone presenting a consistency greater than that of its central part. This zone is called *ectoplasm*, or *ectoplas-*

mic layer, or *plasmalemma,* in opposition to the rest of the cytoplasm which is designated as *endoplasm.* This is the only membrane which exists in the plasmodium of the Myxomycetes, in the Myxamoebae, as well as in various zoospores and spermatozoids of the algae and fungi. In other material, especially in the lower organisms, the equilibrium forms of the protoplasm when in the presence of water consist of lobes or pseudopodia, irregular and changing, blunt or spiny, perhaps in accordance with temporary and local modifications of surface tension. There again everything goes on as if there were an elastic layer around the cytoplasm. Figure 8 represents the division into two tiny plasmodia of *Vampyrella.* The individuals in the process of separation remain for a long time united by a protoplasmic strand which becomes increasingly thin. Then suddenly it breaks in the middle and the

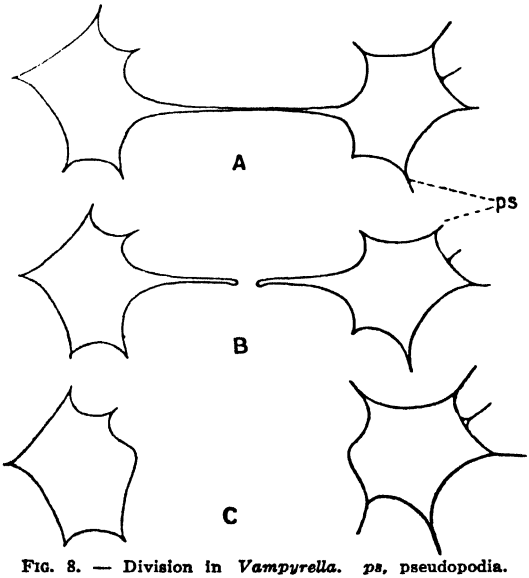


FIG. 8. — Division in *Vampyrella. ps,* pseudopodia.

two points liberated by the rupture go back into the plasmodia to which they belong as if pulled back in by the contraction of an elastic membrane. In all other cells it is recognized that an analogous membrane lines the cellulose wall. When a plant cell is plasmolyzed, for example an epidermal cell of *Iris germanica,* the cytoplasm leaves the cellulose wall and contracts in the middle of the cellular cavity to a perfectly delimited spherical mass as if it possessed a delicate external membrane.

Following work by DE VRIES on the osmotic phenomena of plant cells, there was often given to the region of demarcation between the cytoplasm and the surrounding environment, the value of a differentiated membrane to which the semi-permeability of the cells has been attributed. In no case, however, can this membrane be detected morphologically by any kind of staining. Therefore its existence as a differentiated membrane is purely theoretical

(CHODAT and BOUBIER, LEPESCHKIN). Furthermore, certain experiments are difficult to reconcile with the existence of such a membrane. It has been known for a long time that when a filament of *Vaucheria* is injured in such a way that the torn cellulose wall allows portions of cytoplasm to run out, those coming in contact with water immediately assume spherical form (Fig. 7). They are seen at the same time to become enveloped in a delicate membrane separating them from the surrounding substance.

The microdissection experiments of CHAMBERS on animal cells have demonstrated that the cytoplasm always shows at its periphery a more refractive zone, resulting from a condensation of its substance, constituting a sort of membrane. When trying to insert a microneedle in a living cell, it is noticed that, if the pressure is not too great, there is formed a depression in the cytoplasm at the point of contact as if an elastic membrane separated it from the instrument. But this membrane does not have an autonomous existence. If it is destroyed over a small area, a membrane immediately separates by condensation from the cytoplasm at the wounded spot. By this same method, SEIFRIZ has demonstrated at the periphery of the plasmodium of the Myxomycetes a very elastic membrane, capable of stretching and contracting with the greatest ease, and resistant to a remarkable degree, in short, a limiting layer of more dense cytoplasm. Whenever it is torn, a new one forms immediately, as long as the cytoplasm is alive, whether the surrounding substance be air or water. The same phenomenon is observed in pollen tubes. If the tube is torn, it is seen that the cytoplasmic masses which are detached from it are surrounded forthwith by a membrane. From these experiments it can be concluded that each fragment of naked cytoplasm possesses the property of separating itself immediately, by a delicate membrane, from the substance surrounding it. It is comprehensible that the cytoplasm may be finely divided, being at all times surrounded by a delicate membrane, as, for example, in the plasmodium of *Badhamia* which passed through the cotton wad. It seems demonstrated, furthermore, that this membrane is not, as was first thought, a differentiated membrane of special constitution, but a limiting surface, a sort of physical membrane, brought about by a condensation of the cytoplasm in its zone of contact with the surrounding environment.

The manner in which this membrane is formed can be compared with the well known fact that in a complex liquid all molecules capable of lowering the surface tension accumulate in the peripheral layer. Now, proteins lowering surface tension and concentrating at the surface of their aqueous solution constitute viscous films which reform immediately when torn. It even happens that this concentration at the surface exceeds the maximum solubility of the body in question. It then becomes flocculent and may form a solid surface layer quite comparable to the ectoplasmic membrane.

Certain theoretical considerations, necessary to explain the rapid penetration into the cell of certain substances, brought OVER-

TON, however, to the supposition that the ectoplasmic membrane differed in its chemical constitution from the rest of the cytoplasm and that it was composed essentially of lipides. Other physiologists, for similar reasons, have considered this membrane as a sort of mosaic, formed of lipide and protein particles (NATHANSOHN, CLOWES). The objection raised was that there were no morphological facts to confirm this hypothesis, and that the work of MAYER and SCHAEFFER, as well as that of FAURÉ-FREMIET, demonstrated that the cytoplasm is a lipo-protein complex and that the lipides are therefore scattered throughout all the cytoplasmic substance. There are, however, strong reasons for thinking that the ectoplasmic layer does not have the same chemical constitution as the rest of the cytoplasm but that it differs in being very much richer in lipides. Indeed, in a complex liquid the lipides (lecithin, phytosterol, etc.) which act more strongly on surface tension than proteins, must, according to GIBBS' law, accumulate more at the surface. This constitutes an argument in favor of the existence of an ectoplasmic layer richer in lipides than the rest of the cytoplasm (NIRENSTEIN, HANSTEIN, CZAPEK). The work of RAYLEIGH, DEVAUX, and LANGMUIR have shown that oil put into water comes to the surface where it forms a thin layer, and that its molecules have an orientation which brings about an electrical polarization, perhaps comparable to that which is encountered in the ectoplasmic layer.

Physiological properties of cytoplasm:- Protoplasm is *irritable.* According to SACHS' definition, *irritability* is the capacity of living organisms to react in certain ways under the most diverse influences of the external world. This very complex characteristic is qualified as *physiological* or *biological* because until now its physicochemical mechanism has not been known. It is manifested by specific reactions dependent upon the differences between cells. This characteristic is common to all the morphological constituents of the cell (nucleus, chondriosomes), but is especially easy to observe in the cytoplasm itself. One of the most wide-spread reactions of cytoplasm and the easiest to perceive is *motility* or *contractibility, i.e.,* the property by which the cytoplasm changes from place to place.

This quality can be observed in the Myxamoebae and the plasmodia of the Myxomycetes where the cells lack walls. Here motility is manifested by changes of form and by displacements. Other cells possess permanent extensions of the cytoplasm in the shape of flagella or vibratory cilia which execute movements of rotation and oscillation and act as locomotor organs (zoospores and antherozoids). Besides these external movements, the cytoplasm usually shows internal movements, as discovered in 1774 by CORTI and later observed by TREVIRANUS. These movements are called *rotation, cyclosis* or *circulation* of the cytoplasm, and have been already mentioned in these pages. They are found in most plant cells having walls, where they are the only manifestations of cytoplasmic motil-

ity. They are very easily observed in the epidermal cells of the membranaceous bracts of *Iris germanica* and in the bulb scales of *Allium Cepa.* These movements are observable because of the rapid displacements of the lipide granules contained in the cytoplasm which are seen circulating in the trabeculae between nucleus and cytoplasm. The chondriosomes also are carried in the current but much more slowly. Very favorable objects for the study of these movements are to be found in fungi of the genus *Saprolegnia* and in the leaf cells of *Elodea canadensis.* In *Saprolegnia,* as well as these movements of circulation revealed by the displacement of lipide granules and chondriosomes, there are also sometimes observed displacements of an entire portion of the cytoplasm which carries along with it the nucleus and vacuoles, a movement which seems to be due to a general contraction of the cytoplasm. These movements can be easily observed in moist-chamber cultures. The observations of LAPICQUE, and more recently of MANGENOT on *Spirogyra,* have made clear the general nature of these movements. In direct light, the cells of this alga show a hyaline cytoplasm which, in addition to the ribbon-like chromatophore which will be discussed later, contains, in suspension, chondriosomes in the form of granules and short rods. These are arranged in rows and move toward one extremity of the cell or the other. The chondriosomes of neighboring veins may move in the same or opposite directions, then may mingle in great agitation and soon after disperse, either by all going in one direction or by different chondriosomes taking different directions. These chondriosomes are being carried by currents which agitate the mass of cytoplasm, currents similar to those of the plasmodium and which, like them, appear to be rapid and restricted. These currents form little adjacent veins comparable in certain ways to the convection currents which agitate a liquid as it is being heated. In observing the deeper regions of the cell, the nucleus is seen at the center attached to the walls by several strands of cytoplasm. This cytoplasm lines the internal face of the wall as a thin layer surrounding the vacuole. The boundary between the cytoplasm and the vacuole changes constantly: for instance, the moving cytoplasm accumulates at one point forming a protuberance which immediately disappears while another appears a certain distance away, then is absorbed, and so on.

All these currents are accelerated in the presence of different agents (electricity, various chemical substances, etc.) and are halted by anæsthetics.

The cytoplasm with regard to vital dyes and fixed preparations:- The cytoplasm is permeable to water but as long as it is in the living state, it is little permeable to certain substances dissolved in water. It is often said that cytoplasm is impermeable to most stains. It has been known for some time, however, that certain basic dyes, called vital dyes, such as neutral red, cresyl blue, Nile blue and methylene blue, have the property of penetrating the

cytoplasm of living cells, but these dyes traverse the cytoplasm without coloring it and accumulate exclusively in the vacuoles. They stain the vacuoles only in the living state and disappear in them with the death of the cells, reappearing in the cytoplasm and the nucleus. They will be discussed later (p. 131) in connection with the vacuoles. Some of these dyes, however, such as Nile blue and methylene blue, can at the same time produce diffuse staining of the cytoplasm, especially when used at a high *p*H.

It is shown today, by the work of KÜSTER, confirmed by ours in collaboration with GAUTHERET, that, although some dyes never pass through the ectoplasmic layer, without any reason for this being evident, many others can more or less easily penetrate living cells. Basic dyes are in general the only ones which penetrate the cell. Like those already mentioned, they may accumulate exclusively in the vacuoles under certain special conditions or else may show a predilection for the chondriosomes which they stain first. Most of these dyes end by staining the cytoplasm and the nucleus a little before the death of the cell. Among these dyes, chrysoidine stains the cytoplasm and nucleus superbly in cells which are unquestionably alive, clearly showing cytoplasmic currents. This is without doubt explained by the nature of this dye which is readily dissolved in lipides.

The acid dyes in general do not penetrate living cells. Eosine and erythrosine, however, may color the living cytoplasm and nucleus as KÜSTER has shown. But these dyes, incorrectly considered by this observer as the best suited to staining of the cytoplasm, penetrate living cells only with great difficulty and only a short time before the death of the cells, without doubt because of a modification of permeability produced at that moment. Aurantia, another acid dye, penetrates living cells very slowly but immediately kills them.

It should be noted that all dyes capable of producing vital staining of the cytoplasm do so only between slide and cover glass, therefore in cells placed under defective conditions, and it may be admitted that this is sublethal staining, *i.e.*, staining which occurs only in the period which precedes the death of the cells.

If plants are cultivated aseptically in media to which these dyes have been added, it is noted that the plants grow but no coloration of the cytoplasm is produced. The stains accumulate in the vacuoles. It is only in cells where growth has stopped that the cytoplasm, nucleus and chondriosomes may show staining.

Chrysoidine, for example, which so easily stains the cytoplasm and nucleus in cells placed between slide and cover glass, accumulates only in the vacuoles in plants cultivated in a medium to which this dye has been added, and is scarcely taken up by the cytoplasm except in cells where growth has stopped. Recent research (GUILLIERMOND and GAUTHERET) makes it possible to explain this difference in behavior. The staining of the cytoplasm between slide and cover glass is, in reality, obtained under abnormal conditions and usually with toxic quantities of the dye. This is, therefore, unques-

tionably sublethal staining. Nevertheless in cultures to which vital dyes have been added, staining of the cytoplasm is obtained but this staining is purely transitory and is visible only in the earlier hours of culture. The dye, first taken up by the cytoplasm is rapidly excreted into the vacuole which appears to be the region of the cell in which all toxic products accumulate. It is only when the dye has accumulated in the vacuole that the cell begins to grow and multiply. Consequently, only vital staining of the vacuole is compatible with growth and any persistent staining of the cytoplasm is necessarily sublethal. The same is true for dyes which stain the chondriosomes. The acid dyes, eosine and erythrosine in particular, do not in general stain plants cultivated in media which contain them.

It seems likely that, ordinarily, living cytoplasm does not have an affinity for dyes. Cytoplasm in healthy cells stains only under special conditions or in cells which have ceased to grow. More often, staining takes place just before their death in cells which are not healthy. In the yeasts, however, Nile blue first accumulates in the cytoplasm and is then excreted into the vacuoles. In any case, these dyes, interesting from the point of view of cellular permeability, are of no service in a cytological study of the cytoplasm except as they stain the chondriosomes.

Once dead, the cytoplasm becomes, on the other hand, very permeable to all substances dissolved in water. This can be explained by considering that when the cytoplasm is coagulated, the micelles crowded at the periphery, forming the ectoplasmic membrane, move away from each other and allow the substances dissolved in the surrounding medium, especially all the dyes, to pass through freely.

Consequently, for the study of the cytoplasm, the method of fixation and staining has been resorted to because observation of living material does not suffice. Since the cytoplasm seems to behave like a hydrogel which is very slightly alkaline, as will be shown farther on, its fixation, *i.e.*, its coagulation, can be brought about only by acid dehydrating reagents. The acid reagents usually used are composed of weak acids (acetic, picric), or even strong acids (nitric, trichloracetic), suitably diluted and associated, in most fixatives, with salts of heavy metals (mercury, platinum, osmium). Such fixatives have a *p*H inferior to 3. Besides these, neutral liquids like alcohol and formalin[1] may act as fixatives, for both have the property of precipitating the proteins, the former by its dehydrating action, the latter by combining with the protoplasm to form insoluble compounds still not well defined.

The most acid fixatives, those with an acetic acid base and fixatives containing alcohol, which has a too strong dehydrating action, usually cause sudden coagulation, producing in the cytoplasm artificial structures - coarsely granular-reticulate - which, as will be seen, led the early cytologists to attribute to the cytoplasm a reticu-

[1] Formalin is usually acid, almost always containing, furthermore, some formic acid.

late or alveolar structure. These fixatives, furthermore, have the disadvantage of dissolving the lipides, which are among the essential constituents of the cytoplasm. Reagents containing acid in a very dilute state, *i.e.*, the least acid fixatives, especially formalin, act more gently. They transform the cytoplasm into a very finely granular coagulate which preserves, in appearance at least, the homogeneous aspect it has in the living state.

In general, fixed cytoplasm stains only with acid anilin dyes (eosine, erythrosine, light green, etc.). This distinguishes it from the nucleus which stains with basic anilin dyes. The cytoplasm for this reason is said to be *acidophilic* while the nucleus is *basophilic*. This affinity of the cytoplasm for acids is generally attributed to the fact that in the nucleo-proteins which compose it the nucleic acids may be entirely saturated with simple proteins. These nucleo-proteins thus are presumed to act as bases in regard to the acid dyes.

The use of the method of staining material after fixation has led many cytologists, contrary to the now classic description of DUJARDIN, to believe in the existence of a special organization in the cytoplasm. So for a long time the cytologists endeavored to investigate this structure, either by fixed and stained preparations or by direct observation of living cells, but they encountered two great obstacles. By fixing the cells, they completely upset the constitution of the cytoplasm which, as will be seen, is in a colloidal state and coagulation images were induced. In the second place, by tearing off the epidermis or by sectioning or cutting the tissue in artificial media in order to observe living cells, alterations of the cytoplasm were caused leading to its death.

Cytologists who studied it did not, consequently, agree on the structure of the cytoplasm and numerous theories were proposed of which for historical interest, the principal ones will be summarized here and in as brief a manner as possible:

1. — *Reticular theory*, according to which the cytoplasm is composed of a network of anastomosing filaments immersed in a substance which is more fluid and less refractive (HANSTEIN, REINKE, STRASBURGER, CARNOY, etc.).

2. — *Filar theory*, according to which the cytoplasm is composed of a fluid mass in which are immersed filaments of more solid material which are isolated one from the other (FLEMMING, HABERLANDT).

3. — *Alveolar theory*, which seems to have had the most adherents and which is still advocated by some cytologists. According to this theory, the cytoplasm is formed by an assemblage of small alveoli pressed one against the other with walls which are more compact than the more fluid substance within (BÜTSCHLI, CRATO).

4. — *Emulsion theory* of KÜNSTLER, according to which the cytoplasm is composed of an infinite number of very small protein spheres with compact envelopes and semi-fluid contents. Accord-

ing to this theory, these spheres and not the cell, constitute the essential morphological units of living matter.

5. — *Granular theory* of ALTMANN which assumes that the cytoplasm is composed of a very great number of small bodies in the shape of grains called *bioplasts*, isolated or united in small chains, having the appearance of bacteria. These grains are contained in a homogeneous ground substance. According to ALTMANN, these bioplasts are morphological and physiological units capable of division and of leading an independent life. They are homologous to bacteria which themselves represent independent bioplasts.

Let it be added that some cytologists used to think that all these structures might be encountered in a single cell and represented, in consequence, only functional states of the cytoplasm. Yet the research of HENNEGUY, SCHWARZ, A. FISCHER, CHODAT, etc., had shown much earlier that different fixatives produced as many different structures and that most of the structures described in the cytoplasm were only artifacts produced by the fixatives.

Since 1908, the use of new fixation techniques called *mitochondrial*, producing less violent coagulation and a better conservation of the lipides of the cell, has led cytologists to return to the old idea of DUJARDIN and to conclude that the cytoplasm is homogeneous but encloses in suspension small elements called chondriosomes. Observations of FAURÉ-FREMIET on living cells of Protozoa (1910), ours in plant cells (1913, 1919) and, finally, observations completed on Metazoan cells by using tissue cultures (M. R. and W. H. LEWIS, G. LEVI, etc.) have confirmed these results and shown that the cytoplasm, examined under favorable conditions, always appears as an homogeneous and translucid substance, containing in suspension numerous, slightly more refractive chondriosomes. While this work was being done, the use of the ultramicroscope had led physiologists to conclude, even as early as 1904, that the cytoplasm is in a colloidal state and in 1908 MAYER and SCHAEFFER were able for the first time to show that this cytoplasm always appears *optically empty* under the ultramicroscope and presents the character of a fluid hydrogel. From that time on, therefore, there could no longer be any question of any structure in the cytoplasm other than that inherent in its physical constitution.

All theories proposed for the structure of cytoplasm are therefore out of date today and are now of historical interest only. It is easy to understand how fragile the cytoplasm is, now that its colloidal state is known. Most fixatives bring about the disorganization of the chondriosomes by dissolving their lipides at the same time that they cause a coagulation of the cytoplasm. This coagulated state appearing as a network was responsible for the formulation of the reticular theory. On the other hand, observation of living material, necessitating most often cutting or tearing of the tissue which is then examined in an artificial medium or under imperfect conditions, produces a disturbance in the very delicate

equilibrium of the colloidal system which we now know cytoplasm to be, and brings about more or less important alterations. It has been demonstrated that the spherical and alveolar structures are in reality the result of alterations of the chondriosomes which swell and are transformed into little spheres and large vesicles. As for the filar and granular theories, it will later be seen that they rest on facts exactly observed but wrongly interpreted. Since the proposal of these hypotheses, our knowledge of the cytoplasm has been greatly enriched by observations of the following types of material: fungi observed in their medium of culture, leaves of aquatic plants examined also in their own medium, membranaceous bracts of *Iris* preserved from all alteration by their impermeable cuticle, and organs of plants growing in Petri dishes.

It is thus definitely demonstrated at the present time that the cytoplasm is a homogeneous colloidal system. Its physical characteristics will be studied further on. The method of fixation and staining can not be used in the study of the cytoplasm itself, for this gives only pictures of coagulation which furnish no idea whatever of its actual nature. However, modern cytological work carried out either by special techniques called *mitochondrial* or by direct observation of living cells, with or without vital staining, has revealed the constant presence, in the cytoplasm of each cell, of a chondriome and a system of vacuoles, constituents of which we have already spoken and which will be the subject of the following chapters.

Chapter IV

THE CHEMICAL CONSTITUENTS OF CYTOPLASM

Proximate analysis of the cytoplasm:- It is impossible to analyze the cytoplasm. Only the analysis of the protoplasm as a whole, including the nucleus, can be obtained. When it is a question of specifying the chemical constitution of the cytoplasm itself, we can only have recourse to microchemical reactions which verify the results obtained by proximate analysis but which can give only a very inaccurate idea of the chemical constitution of the cytoplasm.

It is especially the proximate analysis of protoplasm which will give us an idea of the chemical constitution of the cytoplasm. But before discussing the principal results obtained in this field, it is important to stress the exceptional difficulties attending work of this type. At the present time, we possess only analytical processes with which to study the chemical constitution of living matter. Now, protoplasm is killed by the least analytical attempt and one is immediately reduced to working on dead matter, *i.e.*, on a system quite obviously modified. As for the synthesis of protoplasm, living or dead, it has never been realized and in the present state of our knowledge it even seems impossible of realization. What is more, it is not known even how to make the principal substances entering into the composition of living matter — the proteins or lipides, which will be discussed later — or even how to make the very numerous organic products, such as starch or cellulose, which are generally manufactured in the cytoplasm.

Now as BERTHELOT has said, "To know really the nature of things, it does not suffice to destro them. We must be able to recreate them". Analysis teaches how a body may be torn down. It does not indicate how it has been built up. Everything indicates, on the contrary, that decomposition in most cases does not follow in reverse direction the same steps as synthesis. The two processes differ essentially in every respect from each other. These considerations will make it possible to give the proper weight to the data which we now possess on the chemical constitution of living matter.

The plasmodia of Myxomycetes are here again particularly favorable objects for analysis of living matter. They are made up of naked protoplasm, *i.e.*, there is no trace of those more or less thick, always important, external envelopes which living matter secretes at its surface in almost all organs. The plasmodium of Myxomycetes is, therefore, the most directly and easily accessible protoplasm. Several analyses have been published, that, for example, of LEPESCHKIN in 1923 for the plasmodium of *Fuligo septica* which follows:—

Analysis of original (fresh) material			
Water		82.6%	
Dry material		17.4%	
		100.0%	
Analysis of dry material			
Substances soluble in water			
Protides[1]	26.5%		
Sugars	14.2		
			40.7%
Substances insoluble in water			
Protides			
Nucleoproteins	32.3%		
Nucleic acids	2.5		
Albumins	2.2		
Globulins	0.5		
Lipoproteins	4.8		
		42.3%	
Lipides			
Glycerides	6.8%		
Sterols	3.2		
Compound lipides	1.3		
		11.3%	
Mineral salts		2.2	
Miscellaneous		3.5	
			59.3
			100.0%

A study of this table admits of the following conclusion: the protoplasm of the plasmodium of *Fuligo* is composed essentially of proteins in the proportion of 68.8% (including the lipoproteins) and of lipides in the proportion of 15% (including the lipoproteins) and in addition, sugars (14.2%) and mineral salts (2.2%).

The chemical constituents of cytoplasm. Protides[2]:- The protides comprise the proteins and their hydrolysis products, the amino acids. The protides are the essential constituents of cytoplasm and are, as is known, the most complicated of organic compounds. Their molecules are the largest. These proteins characterize living matter which alone has the power to create them.

Proteins are classified in two groups according to their chemical constitution and some of their properties: the holoproteins[3] and the heteroproteins[4]. The holoproteins are made up only of amino

[1] *Cf.* Footnote[3].

[2] ***Translator's note.*** Protide is a more comprehensive term than any used in the classification recommended by the Physiological and Biochemical Committees on Protein Nomenclature, J. Biol. Chem. 4:XLVIII-LI (1908) and refers to proteins and their hydrolysis products, amino acids, amines, amides, soluble proteins, etc.

[3] ***Translator's note.*** Simple proteins. The term *protein* is more generally used by American chemists today than *proteid* and will be adopted here in translation of *protéide* in the French text.

[4] ***Translator's note.*** Conjugated proteins.

acid groups and the hydrolysis of these holoproteins results only in amino acids and their derivatives. The heteroproteins are much more complicated and are divided into nucleoproteins, phosphoproteins and glycoproteins. The heteroproteins give not only amino acids by hydrolysis but other substances belonging to different organic groups.

The nucleoproteins may be considered as the combination of a simple holoprotein and a nuclein, which is itself a combination of holoprotein with a nucleic acid. Nucleic acids are esters of phosphoric acid containing, side by side with this acid, a sugar represented in plants either by a hexose or a pentose (ribose) as well as organic bases of the purine series (guanine, hypoxanthine, adenine) and of the pyrimidine series (cystosine).

The phosphoproteins are proteins which, like the nucleoproteins, give on hydrolysis, amino acids and phosphorus-containing substances other than nucleic acids. As for the glycoproteins, they decompose by hydrolysis into proteins and sugar. The phosphoproteins and the glycoproteins are, moreover, very imperfectly known.

Holoproteins are always found in protoplasm but they seem to play only a small part in the constitution of living matter. They usually represent products of cellular metabolism and are chiefly localized in the vacuoles where they constitute reserve products (aleurone). The greatest proportion of the constituents of living matter seem to belong to the heteroproteins, among which the nucleoproteins dominate. They do not seem to be exclusively localized in the nucleus, contrary to an opinion often accepted, but they are found also in the cytoplasm. In 1881, REINKE and RODEWALD, studying the chemical composition of the plasmodium of *Fuligo septica,* concluded that it is in large part made up of a phosphorus-containing protein presumably formed by the union of a nuclein and a protein. These results assigning to the cytoplasm a nucleoprotein constitution have since been verified in material differing greatly from the above. In 1892, HALLIBURTON observed that the nucleoprotein extracted by him from the kidney was in too great quantities to have come from the nuclei alone and concluded categorically that this protein came above all from the cytoplasm. Then in 1895, HALLIBURTON isolated another nucleoprotein from mammalian red blood corpuscles which do not have nuclei. These analyses and many others published since, that of LEPESCHKIN for example, actually indicate, it would seem, that the histochemical proteins are nucleoproteins. It is admitted, however, that there is a difference between the nucleoproteins of the cytoplasm and those of the nucleus. In the former the nucleic acid is completely saturated by the protein base while in the latter the saturation is not complete and there is some uncombined nucleic acid. The capacity of the nucleus to be stained with basic dyes is due, according to this theory, to the uncombined nucleic acid which becomes affixed to the basic dyes. So it would be explained that the nucleo-

proteins of the nucleus are basophilic while those of the cytoplasm are acidophilic.

Lipides:- As is known, lipides include substances of very varied chemical constitution which fall into one group by reason of their common properties: solubility in ether, chloroform and benzene as well as diverse histochemical characteristics.

Lipides, but for rare exceptions, seem to be exclusively contained in the cytoplasm. Analysis of plant tissues shows that they constitute a considerable proportion of the cytoplasm, 15-25%, and among these are the simple lipides (*lipides ternaires*) and the *compound lipides*. The *simple lipides*[1] include on one hand the gluceride or true fats, esters of glycerol and a fatty acid, and on the other, the sterols[2] composed of alcohols of high molecular weight which can be esterified by fatty acids. The *compound lipides* are subdivided into *phospholipides*, esters resulting from the combination of an alcohol, inositol, with phosphoric acid and the *phosphoaminolipides*, represented by lecithins, which like the glycerides, are esters of glycerol but contain nitrogen and phosphorus and in which two alcohol linkages of glycerol are combined, each to a molecule of fatty acid, the third being united to a molecule of phosphoric acid itself linked to choline and sometimes to betaïne, a substance possessing both the alcohol and the amino function.

A considerable proportion of protoplasmic lipides represent reserve products which accumulate at certain periods in the life of the cells and are later consumed. Such is the case for most of the glycerides and perhaps also for certain lecithins. Sterols and lecithins are also found in the vacuoles where they are products of metabolism whose rôle is not yet known. But the work of MAYER and SCHAEFFER on animal cells, the results of which certainly apply to plant cells, has shown that a notable part of these lipides represent an essential constituent of the cytoplasm. They are, therefore, an integral part of living matter.

These lipides are sometimes refractive globules in the cytoplasm (Fig. 9), or they are sometimes represented in the plastids and chondriosomes but it seems as if a considerable proportion of them were combined with the cytoplasmic proteins as compound lipoproteins. It is difficult to detect them, for, being combined with the proteins, they are invisible and constitute what is known as masked lipides. Their existence may be demonstrated either by histochemical analysis of certain tissues or by chemical analysis.

It is by this chemical analysis that notable quantities of lipides have been revealed in tissues which do not show any in the state represented in Figure 9 and in which no trace of them is found with ordinary histochemical methods. Among these masked lipides there are some, however, which are united to the proteins in an unstable

[1] The translator thinks these substances are probably complex as they contain both the compound and derived lipides of BODANSKY's classification. BODANSKY, M. Introduction to Physiological Chemistry. Wiley and Sons, New York, 1938.

[2] *Translator's note.* The sterols actually occur partly as free alcohols and partly as esters of fatty acids.

way, in weak chemical combination or by a simple physical adherence. These may be disclosed by special histochemical methods isolating them from their combination, *i.e.*, they may be unmasked. Others of these masked lipides are united as stable chemical compounds in a very intimate manner with the proteins and can be demonstrated only by destroying the protein substances to which they are united, either by chemical hydrolysis or by digestion. Chemical analysis, therefore, is the only process by which their presence can be demonstrated.

It would seem as if this would hold true for plant cells. It has been observed that tissues rich in proteins are also rich in lecithins. It is also probable that lecithins are among the most important chemical constituents of the plastids and chondriosomes. Some authors, having ascertained a fixed relation between the content of lecithin and chlorophyll in tissues, state that this pigment exists in the chloroplasts, in a combined state, as chlorolecithin.

Lipide granules which are found in all cells are perhaps the product of an unmasking of lipides which takes place normally under certain conditions. This phenomenon seems to be more pronounced during cellular degeneration resulting in fatty degeneration (*lipophanerosis*).

It must be added that DUJARDIN had observed that when the cuticle surrounding the cellular body of certain Infusoria is injured, the cytoplasm in contact with water forthwith forms droplets which he calls "boules sarcodiques", balls of sarcode. MAGGI, who later took up the study of them, thought that they corresponded to the figures observed when myelin (a compound lipide forming the peripheral layer of axone in higher animals) comes in contact with water. There are found then, at the surface of the myelin, filamentous protuberances whose extremities finally swell out in the shape of clubs. Many cytologists have found similar figures (KÖLSCH, ALBRECHT, SCHNEIDER, PROWAZEK, FAURÉ-FREMIET, KÜSTER) both in animal and plant cells. These figures form in large quantities all around the vacuoles during the phenomena of plasmolysis. They form pedicelled buds which may break off, and become introduced into the vacuole as vesicular buds and might correspond to the myelin figures arising by separation of the lipides from the cytoplasmic lipoprotein compound.

Various products and mineral substances:- It has been seen that analysis reveals in the cytoplasm, but in smaller quantities, other products of different sorts: sugars resulting from cellular metabolism, localized mostly in the vacuoles, and, more important, mineral substances, among which potassium, magnesium, iron may be especially mentioned. These minerals which seem to play a very important rôle in the manifestations of living protoplasm, may be in the form of ions or molecules or may be fixed by adsorption to protein substances and enter into the cytoplasmic lipoprotein complex.

Water:- Water is an important constituent of protoplasm. In fact, all protoplasm contains water interposed between its micelles. This water is indispensable for the manifestations of vital phenomena as these can be produced only if the protoplasm is in a semi-fluid state. The water content of living substance varies with age, *i.e.*, according to the physiological state of the cells and also, in certain plants, according to the environmental conditions.

In a general way, in the course of the life of a plant, the maximum water content coincides with the maximum physiological activity. The differences which exist in the viscosity of the cytoplasm, varying with the age of the cells, have already been mentioned. The amount of water held in the cytoplasm varies from 80-90% by weight. A part of this water may be removed but then the vital activity is diminished. If it is almost all suppressed, the protoplasm in certain cases may not die but passes into a state of retarded activity (*anhydrobiosis*). This is the case normally in the spores of bacteria and fungi, as well as in the seeds of phanerogams which, during maturation, become dehydrated until they contain only 10-18% of water and sometimes, as in certain fatty seeds, as little as 5-6%.

Chapter V

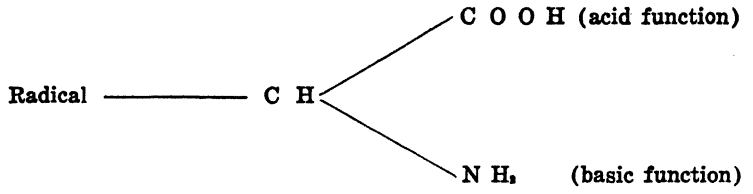
PHYSICO-CHEMICAL CONSTITUTION OF THE CYTOPLASM

The cytoplasm, as has been seen earlier, appears to be constituted essentially of proteins (68.8% of dry weight), water-insoluble lipides (about 15% of dry weight), and a large proportion of water (80-90% of fresh material) containing various mineral substances in solution. It may be considered then as a colloidal solution whose micelles are represented by protein molecules united with lipide molecules, the intermicellar liquid being water.

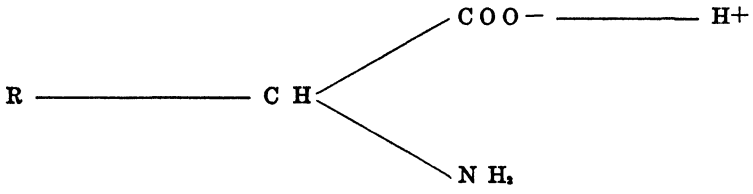
It seems that every cell contains the same chemical constituents and that differences between the cellular types exist only in the proportions of the chemical constituents present. One is obliged to admit, however, that each type of cell has a protoplasm, and therefore a cytoplasm, which is peculiar to it. The widely-varying properties of the plastids are identified not only by the differences of minimum and maximum temperatures necessary for their growth, but by the elaboration products of their protoplasm, which differ essentially from one cell to another. The diverse properties of different plants are due to the individual constitution of their protoplasm and, in particular, of the cytoplasm which is peculiar to each. One is therefore driven to the idea of specificity of protoplasm but chemical analysis has not so far furnished any basis for this notion. Nevertheless, the possible number of different nucleoproteins being theoretically indefinite, it is admissable that each species of animal or plant be characterized by a special nucleoprotein.

Electrical characteristics of proteins:- The work of MICHAELIS, PROCTER, WILSON, and especially of JACQUES LOEB, confirming an hypothesis formulated a long time ago, has demonstrated that the proteins belong to the class called electrolytic colloids which behave as ionisable electrolytes. Their micelles, seeming to correspond to enormous molecules, ionize to form true micellar ions, or protein ions, having an electric charge analogous to that resulting from the dissociation of the valence bonds of an ordinary electrolyte.

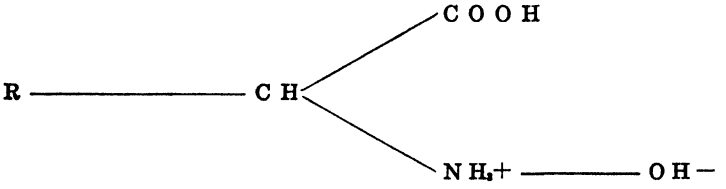
The proteins correspond to electrolytes called *ampholytes*, that is to say, possessing at the same time acid and basic functions. They are formed, as is known, of amino acids combined one with the next, a great number of times, to form enormous molecules. Now an amino acid may be schematically represented by the following formula:



There is, therefore, an acid function COOH and a basic function NH_2 and this amino acid is an electrolyte in both senses, being able by dissociation to give an H^+ ion as well as an OH^- ion. In the first case there will be:



and with the addition of one molecule of water, in the second:



When the medium is acid, the dissociation of the acid function is diminished or blocked by virtue of the law of mass action, whereas all the OH^- ions leave the radical of the ampholyte and the basic function is manifested. The ampholyte, then, is composed only of $R \cdot H^+$ and behaves like a cation. In the electric field it will migrate to the negative pole. When, on the contrary, the medium is basic, the dissociation of the basic function diminishes or is blocked, whereas that of the acid function increases. The ampholyte is now constituted only of the anion $R \cdot OH^-$. It behaves like an anion and migrates to the positive pole.

Finally, and this is very important, for a certain intermediate reaction between the two extremes, the dissociation of the acid valence being equal to the dissociation of the basic valence, the ampholyte is both an anion and a cation simultaneously and therefore behaves somewhat as though it did not have any electric charge, and does not move in the electric field. This point of no migration is called the *isoelectric point.* It is important to notice that this isoelectric point does not generally correspond to chemical neutrality but to a particular *p*H (isoelectric *p*H indicated by the symbol *p*Hi).

At their isoelectric point, the ampholytes show, as has been demonstrated by JACQUES LOEB, a series of special properties: a

minimum of solubility, a minimum of viscosity and of linkage with peripheral electrolytes (*i.e.*, minimum ability to combine), minimum swelling in water, maximum rigidity and maximum instability in solution. At the isoelectric point the ampholyte is extremely apt to flocculate. It seems therefore that the fundamental properties of cytoplasm must be dominated on the one hand by the *p*H of the intracellular liquids and on the other hand by the isoelectric point of its constituents.

Physical constitution of cytoplasm:- The classical researches of MAYER and SCHAEFFER (1908) made an important contribution to the study of the colloidal state of cytoplasm in so far as animal cells are concerned. These results apply equally well to plant cells and must be considered first.

MAYER and SCHAEFFER undertook first the study, with the ultramicroscope, of colloidal solutions of different organic colloids. They then in a comparative way were able to begin the study of animal cells. They showed that these solutions may present two very different optical appearances. Some, such as glycogen, dialyzed albumin, and diastases, are characterized by a suspension of a large number of micelles, brightly lighted and animated by Brownian movement. These are the *hydrosols.* Others, such as white of egg and nucleoproteins, appear optically empty and without any visible micelles whatever. They are filtered with difficulty and show very high viscosity. They are composed of micelles containing a great deal of water, thus possessing a strong linkage with the intermicellar liquid, *i.e.*, water, so that the micelles do not refract light and do not appear in the ultramicroscope. To these latter liquids, MAYER and SCHAEFFER give the name *fluid hydrogels.* Under certain influences, by hydration of their micelles, these hydrogels can be transformed into hydrosols. They appear as confused luminous streaks — this is called the *non-resolvable nebulous stage* — then as very fine granules which agglomerate progressively and the field becomes completely starred with brilliant dots. This is the *resolvable nebulous state i.e., flocculation.* This reversible phenomenon may be succeeded by a more important and irreversible modification, *coagulation,* accompanied by physical and chemical phenomena which finally agglomerate the particles into a single mass.

So MAYER and SCHAEFFER set off against each other two different physical states: the *hydrosol,* appearing in the ultramicroscope as a suspension of micelles vividly lighted and animated by Brownian movement, a state similar to that presented by pseudosolutions of metals; and the *hydrogel* appearing as an optically empty pseudosolution which is very viscous. These investigators designate by the name *jelly* the semi-solid state of the hydrogel which is much more viscous and endowed with a very definite rigidity. This terminology is not accepted by all authors. Some writers distinguish the *hydrophobic colloids* (mineral colloids) whose micelles do not have any affinity whatever for water and

form hydrosols, from the *hydrophilic colloids* whose micelles have a strong affinity for water and produce solutions which are optically void (fluid hydrogels of MAYER and SCHAEFFER). Other authors have reserved for this latter group the name *isocolloids* and differentiate between the *semi-fluid state* or *isocolloidal hydrogel* and the *fluid state* or *isocolloidal hydrosol.*

The structure of the hydrogels is, moreover, variously interpreted. Certain authors consider the hydrogel as formed by an assemblage of micelles lying more or less closely together and heavily saturated with water (BRADFORD). Others consider it as constituted of a network holding water in its alveoli (HARDY). Still others including PROCTER, WILSON, JACQUES LOEB, think that there is no difference between a true solution and a protein hydrogel. They believe that the protein hydrogel is made up of a homogeneous dispersion of protein and water molecules, their proportional amounts varying greatly and regulating the consistency of the system. LUMIÈRE, elaborating on this conception, separated into two categories the substances which until then had been designated as colloidal: first, the *micelloid colloids,* in the state of hydrosols, formed of voluminous polymolecular particles and visible in the ultramicroscope and second, *molecular colloids,* characterized by the optically void state of their solution, formed of monomolecular particles and taking on, as they solidify, the aspect of glue. These last, according to LUMIÈRE's view, are in reality true solutions, distinguished from solutions of crytalloids only by the enormous dimensions of their molecules to which they owe their special properties.

DEVAUX, who shared this opinion, looks upon these colloids as allied to strongly polymerized compounds, such as cellulose and rubber recently studied by STAUDINGER, which DEVAUX considers as formed of molecules characterized by being extraordinarily long but still conserving transversely the dimensions of ordinary molecules, *i.e.,* molecules arranged in a line. This, according to DEVAUX, explains their colloidal properties, for example their ability to swell up in certain liquid media and give viscous and ropy solutions. Certain reasons would seem to support this opinion. It is known, in fact, that two molecules of amino acids may combine by peptide bonds and, by repetition of the process, form a long chain constituted of molecules arranged in lines carrying laterally, like oars, the radicals of various amino acids. Several polypeptides may combine in this way to give a very long chain, straight or folded over, such as keratin which, according to the work of ASTBURY, shows linear molecules (X-ray patterns). The knowledge of protein structure is not far enough advanced so that it can be known whether generalization can be made from this structure to include cytoplasmic proteins. Nevertheless, some biologists recognize the existence of the fibrillar structure and crystalline nature of organic gels and in particular of the cytoplasm (SEIFRIZ). This structure would permit them to explain, in accordance with SEIFRIZ's opinion, both the pulsations observed in the plasmodia of Myxomycetes

(COMANDON and PINOY, SEIFRIZ, MANGENOT) and the elastic properties of the cytoplasm.

Utilizing the results obtained from the study of protein solutions, MAYER and SCHAEFFER with the ultramicroscope began the study of animal cells living under favorable conditions. This study permitted the authors to observe that the cytoplasm presents in the ultramicroscope only a few lighted granules, which do not show Brownian movement. They are not to be classed as micelles for they are always visible in direct light. In addition to these granules which belong to the paraplasm, the cytoplasm, like the nucleoprotein solutions, does not show under the ultramicroscope any visible micelle at all. SCHAEFFER, therefore, has

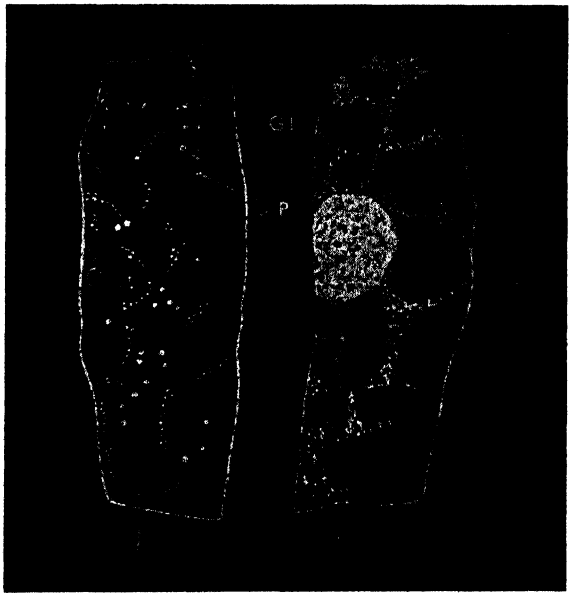


FIG. 9. — Ultramicroscopic view of epidermal cells of the leaf of *Iris germanica*. 1, In the living cell the nucleus is opalescent, the nucleolus and cytoplasm optically empty. The granules correspond to lipide inclusions either (*P*) within invisible filamentous plastids or (*Gl*) dispersed in the cytoplasm. 2, In the coagulated cell the nucleus and cytoplasm are entirely luminous.

identified it with a fluid hydrogel. Now a fluid hydrogel behaves like an electronegative hydrogel. Like all the alkaline or negative gels, it becomes cloudy when it is put into acids: at first confused luminous streaks are seen, then ultramicroscopic granules which soon become visible in direct lighting and assemble in a network. Finally the cytoplasm becomes entirely luminous. It is then *coagulated.* The salts of heavy metals and, in general, all substances employed as fixing agents, act in the same manner as acids, by making the cytoplasm appear granular and vesiculate. The dehydrators (alcohol, heat) act similarly. On the contrary, in the presence of alkalis the cytoplasm remains optically empty.

The ultramicroscopic observations of FAURÉ-FREMIET on Infusoria and sexual cells of the Metazoa, followed by the work of others (AGGAZZOTTI, MARINESCO, MOSSA, etc.), have confirmed the results of MAYER and SCHAEFFER on the optically void state of the cytoplasm. Furthermore, observations on living cells of tissue cultures by LEVI and the micromanipulations of CHAMBERS are equally in accord with these results. It is, however, to be noted that some cytologists, such as CHAMBERS, have designated under the name of hydrosol the state corresponding to the fluid hydrogel of MAYER and SCHAEFFER.

The question of the colloidal state of cytoplasm has been much more discussed in connection with plant cells. The first observers, GAIDUKOV and RUSSO, using the ultramicroscope, came to the following conclusions: The cytoplasm of these cells appears as a heterogeneous structure; its micelles are animated by Brownian movement; the cytoplasm, therefore, offers the characteristics of a hydrosol. This opinion is still held today by LEPESCHKIN. PRICE has stated, on the contrary, that the cytoplasm of plant cells most often appears optically empty, *i.e.*, as a hydrogel. However, this author concedes that it may pass from the state of a hydrogel to that of a hydrosol. PENSA has described in plant cells a heterogeneous structure in a semi-fluid suspension medium, presenting a luminosity always more or less marked. He hesitates to consider it as a hydrogel or a hydrosol. In this substance he believes there is a dispersed and solid phase represented by a few strongly lighted microsomes and a dark liquid phase also containing some microsomes animated by Brownian movement.

The work of LAPICQUE and his students, that of BECQUEREL, and our work have shown, on the contrary, that the cytoplasm of plant cells takes on the same aspect as that of animal cells. It is always optically empty in living cells and becomes entirely luminous when it is coagulated, appearing like snow. Our research has proved that the diverging results of the men cited above are explained by the fact that these workers neglected to make a comparative examination of the living cells with lateral and direct illumination. That which those writers who recognize a heterogeneous structure of the cytoplasm describe as micelles, corresponds to the microsomes or vacuolar precipitates, which will be discussed later. These are not of micellar rank and are observed quite as well with direct light as with lateral illumination. Furthermore, some authors, like PENSA, seem to have carried out their observations under unfavorable conditions and to have described cells in which the cytoplasm was already beginning to coagulate. Observations of living material that we have carried out in the most varied plant cells (epidermal cells of Monocotyledons, filaments of *Saprolegnia* and of other molds, yeasts, etc.), whether with the ultramicroscope or the ordinary microscope, have always shown us the cytoplasm as homogeneous, optically void, and translucent.

Today, therefore, it may be recognized as definitively established that the cytoplasm in plant cells as well as in animal cells

appears optically empty as MAYER and SCHAEFFER described it. What remains still obscure in the question is the physical structure which can be attributed to that optically empty state which characterizes the cytoplasm, both in plant and animal cells. From every point of view the cytoplasm appears as a colloidal solution or a fluid gel. It is not, however, miscible with water. This is a fact of capital importance which distinguishes it essentially from all gels or solutions. To designate the peculiarity of this gel-like system of not being able to mix with the excipient, water, although water represents 90% of its weight, the Italian physiologist, BOTTAZZI, has created the term *gliode*. The gliode is a colloidal system existing exclusively in living cells, particularly in the cytoplasm.

KRUYT and BUNGENBERG DE JONG in recent work have sought to explain this peculiarity by considering the cytoplasm not as a hydrogel but as a coacervate. They have shown that a very slight addition of the precipitating agent may bring about, at first, a partial separation of the colloid from the solution, in the form of a mass retaining a considerable quantity of the solvent and still showing, in consequence, a more or less marked fluidity. This colloid which is fluid but more concentrated than the original system is miscible with an excess of the solvent. This phenomenon is called *coacervation* and the fluid mass, separated as described, is called the *coacervate*. Now coacervate systems are very reminiscent of cytoplasm. Such systems can be prepared in the laboratory. BUNGENBERG DE JONG has been able to produce coacervates in which water, proteins and lecithins, *i.e.*, the normal constituents of protoplasm, are actually associated in artificial cells, presenting striking analogies with real cells and showing a central body, like the nucleus, and globules of oil, separated by invisible films. What is more, these systems are stable when the *p*H value is in the neighborhood of 7.4, *i.e.*, when the *p*H values are comparable to that which seems to exist in the cytoplasm. Of course these resemblances are of a purely physical nature and do not in any way concern the absolutely inimitable physiological activity of living cytoplasm.

Cellular constants and equilibria:- Some substances exist in the protoplasm in greatly varying proportions, depending upon internal or external factors (nutrition of the organ and the condition of the medium respectively). The glycerides are such substances and in the cells constitute transitory deposits which, by their nature, are capable of being mobilized. This is not true of the permanent constituents of the cytoplasm, such as the proteins, lipides and water. These latter show differing properties and keep their differing properties when in the cell. The stability of the cell is maintained only because its various properties are in equilibrium. The permanent constituents are present in the cytoplasm in fixed proportions, independent of external or internal conditions.

MAYER and SCHAEFFER have done some work on animals, the results of which it would seem possible to extend to plants. They

think that the properties of protoplasm are determined by the proportions of its fundamental constituents. These are not distributed in a hit-or-miss fashion, but are always found for the same type of cell in invariable ratios which MAYER and SCHAEFFER call *cellular constants.*

It is thus that the fatty acid content is very variable from one species to the next but for all tissues of a single species it always fluctuates about a constant value.

In the matter of cholesterol, the content differs very greatly from one organ to another in the same species but is rather constant for a given organ no matter what the species. It is characteristic of the organ under consideration. The ratio cholesterol: fatty acid, or the *lipocytic coefficient,* is characteristic of a given species. It constitutes a cellular constant. There exists also a mineral constant. Similarly the water content for each type of tissue always fluctuates about the same value, therefore each type of tissue in a species possesses a constant and specific content of water of imbibition. Water is thus a cellular constant. The research of NICOLLE and ALILAIRE brings out similar results for bacteria. Thus in a typhus bacillus there is found 85% of water whereas in an anthrax bacillus there is only 75%.

The *p*H value, the isoelectric point of cytoplasmic colloids, and the *r*H, the oxidation-reduction potential of cytoplasm, appear also to be cellular constants.

MAYER and SCHAEFFER have shown, furthermore, that there exists for each type of cell a constant ratio in the proportions of these different constants. Thus a close relationship is observed between the ratio of cholesterol: fatty acid, or *lipocytic coefficient,* and the imbibition of water by cells, the higher the coefficient, the more water imbibed by the cells. The lipocytic ratio is thus an index of a cellular property.

This relation between lipocytic coefficient and the water content of a cell is explained in the following manner: cholesterol, as has been said, has the property of absorbing a rather large quantity of water by imbibition. The combined fatty acids, on the contrary, do not imbibe water. But for some time it has been shown that cholesterol and fatty acid compounds are soluble one in the other and this mixture becomes penetrable to water. Thus, the more cholesterol there is contained in a protein gel or coacervate, the more water there will be imbibed.

The outcome of this research is that the cells of organisms do not show their known properties to the same degree. They have general properties which are encountered in all cells (protoplasmic properties) and properties which are related to the function which they perform in the cell. The general properties depend upon constituents whose value appears constant for each cellular type.

Ionic reaction of cytoplasm:- Unfortunately, in spite of their precision, the methods for measuring *p*H are not easily applicable to living cells. What is needed, if it were possible, is a method for

measuring cells which have not been injured, for the very delicate protoplasm modifies its *p*H the moment that there is any alteration in the cell. VLÈS has insisted, on the other hand, on the fact that the measurement has no value unless during the entire experiment the cells keep their normal CO_2 pressure. Now the altered protoplasm in contact with air, unless special precautions are taken, loses its CO_2, which raises its *p*H, and it is difficult in the extreme to realize ideal conditions.

Electrometric methods or Clark indicators are used to determine *p*H values. The electrometric method has been used on crushed cells, extracts of plant juices, and finally on living cells.

VLÈS, REISS and WELLINGER have advocated a technique which consists of crushing the cells after instantaneous freezing and effecting a measurement at the precise moment when the crushed mass returns to the cryoscopic point. In this way all escape of CO_2 seems to be avoided. But more often one is limited to measuring the *p*H of plant juices obtained from crushed tissues by the electrometric method, sometimes even in a pressure of 100 atmospheres (KAPPEN). Another method consists of introducing microelectrodes into living cells (CROZIER, ELLIS, PÉTERFI, SMALL) but this is applicable only in cases of large cells. This method likewise causes injuries to the cells and undoubtedly brings about a mixture of cytoplasm with vacuolar sap. It is moreover a difficult operation in plant cells because of the resistance of the cellulose walls.

As the electrometric method is extremely delicate and necessitates costly apparatus, the method most used is the Clark indicator method. This presents great disadvantages, for the point of color change may be modified by the presence of protein matter and its products of disintegration, such as the amino acids. Thus in colloidal gels the color change is aberrant in consequence of chemical reactions. This is called the protein error. Other causes of error result from the fact that the indicators dissolved in the lipides are not dissociated and that then their color does not correspond to any indicator. This causes a lipide error. The point of color change may also be displaced by the presence of a strong concentration of neutral salts: the salt error. It may also be changed by a modification in the concentration of the indicator: concentration error. Finally, the indicators do not penetrate living cells. VLÈS has used the method of crushing cells which consists of producing a sudden yet careful pressure on cells in an indicator solution between cover slip and slide, causing a break limited to the cell wall. An immediate release in pressure leads then to the taking into the interior of the cell of a small quantity of the indicator, the color of which can thus be noted before any effusion of CO_2.

Botanists, however, have usually been limited here also to the use of indicators either on juices extracted from tissues or on sections of tissues which, in being sectioned, were necessarily injured.

A more precise method for measuring *p*H has been to introduce dyes by micro-injection with the aid of a micromanipulator. The

results obtained by these different methods, that of micro-injection excepted, are very divergent and have resulted in *p*H values ranging from 3.4-5 to 7.8, whereas, according to VLÈS and REISS, as well as according to SMALL, the *p*H is 5.1-6.2. There is reason to wonder as to what is the value of results obtained by these methods. First, no value can be placed on results obtained from plant juices since they do not express the *p*H of living protoplasm, but only inform us concerning that of the vacuolar sap, more or less strongly modified by the treatment. One can not deny, furthermore, that the other processes, that of VLÈS in particular, offer great disadvantages because the crushing practiced on living cells, no matter how rapidly executed, does cause injuries and these lead rapidly to the death of the protoplasm. It seems evident that protoplasm becomes acid immediately after death.

In addition, and this is the real objection, none of these processes allows the total *p*H value of the protoplasm to be obtained without regard to the parts which make it up. Now, among these parts the vacuoles must be considered. As will be seen, they give a clearly acid reaction. It follows that the results obtained have no value, for they are only the *p*H value of the cytoplasm and vacuoles combined, and are obtained, usually, after the death of the cells.

More precise results obtained by micro-injection, with the aid of Chamber's micromanipulator and Clark indicators, have given a *p*H value neighboring on neutrality: about 7— from 6.8-7.2 (D. and J. NEEDHAM, RAPKINE and WURMSER). It is suspected, moreover, that micro-injection itself can induce disturbances in the cell and modify the reaction of protoplasm. The ideal would be to measure the cellular *p*H by means of vital dyes. Unfortunately the Clark indicators do not ordinarily penetrate the cells and are all of them more or less toxic.

Cellular *r*H:- It is possible to measure directly the cellular *r*H in accordance with the oxidation state (colored) or reduction state (uncolored) taken on by the dye in the cellular medium. This is accomplished by micro-injection into the cells of oxidation-reduction indicators. The *r*H value given by cells in the presence of air varies from 12 to 14. The *r*H value of the same cells measured after an anaerobic period is in the neighborhood of 7 (BROOKS in *Valonia*, WURMSER and RAPKINE in *Spirogyra*). The micro-injection method has the disadvantage of injuring the cell and of introducing indicators which are generally toxic. The use of vital dyes appears to be the best method. It is unfortunate that these accumulate almost entirely in the vacuoles and generally produce only sublethal staining of the cytoplasm. However, with GAUTHERET, we have used Janus green. In yeasts, particularly in *Saccharomyces cerevisiae*, this dye is taken up by the cytoplasm and is rapidly reduced there to its rose derivative, which does not revert to its green form. If the medium contains nutrients, the dye is excreted to the exterior in the form of the rose derivative. If these nutrients are composed

only of potassium nitrates (1% at *p*H 9), the rose derivative of Janus grèen will stain the cytoplasm of yeasts and in the absence of air will completely lose its color. It is, therefore, reduced to its leucoderivative. If the medium is aerated, the leucoderivative is oxidized and returns to its rose form. This reduction from the rose to the leucoderivative takes place at *r*H 5.2. This limit seems to be reached at the end of a long time and under experimental conditions. It seems, therefore, to be the lowest obtainable *r*H value. The method is a safe one to follow as yeasts treated in this manner can bud in a very active way after having excreted the dye.

Chapter VI

THE PLASTIDS

The plastids (Fr. *plastes, plastides, leucites*):- It has been known for a long time that chlorophyll is found localized in small bodies which were first observed by MEYEN (1828) and described by him under the name of *chlorophyll grains*. These bodies were further studied by numerous workers on the cells of the phanerogams, where they appear scattered in the cytoplasm as numerous globular or lenticular bodies measuring from 6-10μ in diameter. NÄGELI (1846) first noticed that they multiplied by division. Later, VON MOHL (1838-1856), and then SACHS (1852), established the fact that these bodies are composed of a substratum which is colorless, insoluble in alcohol, protein in nature and which, like the cytoplasm, stains yellow with an aqueous solution of iodine. On this substratum is fixed the chlorophyll which can be dissolved in alcohol, leaving behind the colorless substratum. VON MOHL and SACHS finally proved that these bodies are the seat of starch formation. In experiments, now classical, SACHS succeeded in showing that the starch grains which are formed in the chlorophyll grains are the direct products of assimilation in the presence of chlorophyll. These bodies were later the object of splendid research in the phanerogams by SCHIMPER (1883) and MEYER (1883), who will be spoken of later. These bodies were called *chloroplastids* (SCHIMPER), *autoplasts* (MEYER), *chloroleucites* (VAN TIEGHEM) or *chloroplasts* (ERRERA). It is not necessary to stress the importance of these bodies which are the center of the formation of chlorophyll, starch grains and many other products and which play an essential rôle in photosynthesis.

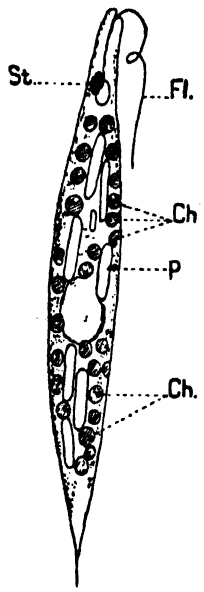


FIG. 10. — *Euglena viridis*. *St*, stigma (eyespot). *Fl*, flagellum. *Ch*, chloroplasts. *P*, paramylum.

The evolution of these chlorophyll bodies in the higher plants, the pteridophytes and phanerogams, remained obscure for a long time. It is known, indeed, that in these plants chlorophyll is not generally found in the egg or in embryonic cells and appears only in the course of cellular differentiation and then only in tissues exposed to the light. In embryonic tissue and in all root tissue, chloroplasts are generally not encountered. The question as to the origin of these bodies was raised but it was not possible to answer it until very much later.

In the algae, chlorophyll is always contained in all parts of the thallus and, in consequence, chloroplasts are found in all the

cells. These chloroplasts sometimes have the same form and dimensions as in the higher plants, but often these organelles are greatly differentiated, of a complex structure and are present in the cell only in very small numbers. Sometimes there is only one to a cell. In that case it is voluminous and generally contains small refractive bodies which are colorless, rounded, or angular, known as *pyrenoids* (Fig. 12). At the surface of these grow the starch grains which form a sort of crown about them. Because of their complex structures the chloroplasts are often designated by a special name, *chromatophores*, but we shall see that they correspond to the small chloroplasts of phanerogam cells (Fig. 10). The life history of these chloroplasts is not difficult to follow and SCHMITZ (1882) showed that they are always transmitted from cell to cell by division, quite like the nucleus. STRASBURGER was even able to follow the division of the single chloroplast in living *Spirogyra* during cell division.

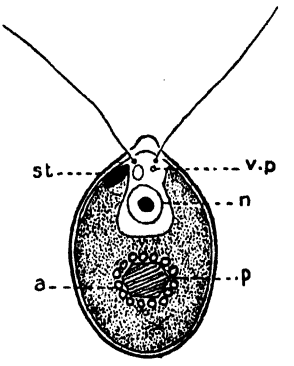


FIG. 11. — Motile cell of *Chlamydomonas* surrounded by a cell wall which forms a beak between the flagella at the anterior pole. Stippled region shows chloroplast. *st*, stigma. *p*, pyrenoid surrounded by *a*, starch. *n*, nucleus. *v.p.*, pulsating vacuole whose rôle is imperfectly known. (× 1650).

The chloroplasts in algae and bryophytes:- The study of the plastids will begin with the algae. In the Cyanophyceae, which are the most primitive of the known algae and whose cellular organization differs from that of the others, there are no chloroplasts. Their cells contain a chromatic network, *the central body*, which occupies the greater part of the cell and which must be considered as a primitive nucleus. Surrounding this is a thin parietal zone of cytoplasm which contains chlorophyll in a diffused state to which is added a blue protein pigment, *phycocyanin*, and sometimes a red protein pigment, *phycoerythrin*. Some authors have called this parietal layer a chromatophore (FISCHER, MEYER), but this opinion seems difficult to justify, since the parietal zone does not in the least resemble a chloroplast and encloses, all around the central body, small vacuoles which can be stained in living tissue with neutral red. As well as the Cyanophyceae, the algal Flagellates (Phytoflagellaceae) are considered to be most primitive algae and are thought to be the ancestors of all the other algae. In these, the chloroplasts in each cell are sometimes numerous, as in many Euglenas, and look like those found in the phanerogams. In other Euglenas there is only one star-shaped chloroplast. In the Peridiniales, which are also classed among the flagellated algae, the chloroplasts may be small, numerous, rod-shaped bodies or may be reduced to a single body appearing as a fine network and occupying the entire cell (CHATTON). In many flagellated algae (Chrysomonadales, Polyblepharidales), however, there is only one bell-shaped chloroplast which occupies one of the poles of the cell, a position

encountered in many zoospores of the Chlorophyceae. In the Diatomaceae, a group often considered as closely related to the flagellated algae, there are a small number of large, plate-like chloroplasts, often only two, occupying a marginal region of the cell.

In the Chlorophyceae, the chloroplasts appear as large organelles of rather complex structure. In the Ulothricales, for example, there is only one chloroplast per cell (*Ulothrix, Draparnaldia*) in the shape of a deeply dentate ring girdling the elongated cask-shaped cell near its middle. In the Siphonales and the Siphonocladiales, the chloroplasts are variously disposed and appear in many different shapes. There may be one single chloroplast per chamber or cell, distributed throughout the cytoplasm in the shape of a network whose numerous swellings are occupied by pyrenoids (*Cladophora*), whereas in algae, such as *Acetabularia,*

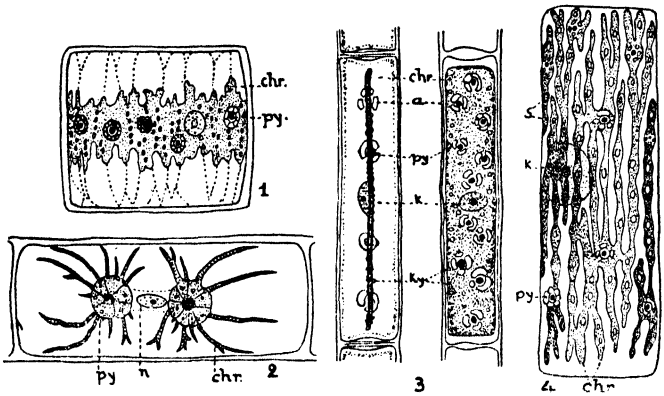


FIG. 12. — Chloroplasts of the Chlorophyceae. 1, *Draparnaldia* (After SCHMITZ). 2, *Zygnema*. 3, *Mougeotia scalaris*. Two views. (After PALLA). 4, *Oedogonium*. (After SCHMITZ). *chr*, chloroplast. *py*, pyrenoid. *n*, *k*, nucleus. *a*, *s*, starch. *ky*, tannin bodies of Palla.

the sections enclose numerous chloroplasts of irregular contours, each chloroplast carrying several grains of carotin but not having any pyrenoids and starch grains (MANGENOT and NARDI). In *Vaucheria,* the chloroplasts, distributed in great numbers in the tubular thallus and never enclosing starch, are bodies of the dimension and form of those found in the phanerogams. They are also small in *Caulerpa* and *Derbesia,* which, although lacking in pyrenoids, seem often to elaborate starch by a rather strange and still imperfectly known process (ERNST, CZURDA).

In the Conjugatae, the chloroplasts are of a very complex form to which particular attention must be called. There are only a few or even only a single one present in each cell. Thus in *Cosmarium* and *Zygnema* there are found in each cell two large star-shaped chloroplasts whose long delicate arms radiate from a pyrenoid situated in the center. In others, *Mougeotia* for example, the single chloroplast is a large smooth plate. A chloroplast of *M. laetevirens* may attain a length of 300μ and a width of more

than 40μ. Each cell of *Spirogyra* contains one or more chloroplasts. Each is ribbon-shaped with slashed edges, rolled into a spiral by more or less tight turns and contains numerous pyrenoids lined up along its median region. *Closterium* contains several large chloroplasts analogous to those of the Diatomaceae. They are plate-shaped and occupy the marginal region of the cell.

In the brown algae or Phaeophyceae the chlorophyll is always associated in the chloroplasts with a brown pigment of the carotinoid group, *fucoxanthin*. Because of this pigment which masks the chlorophyll, the chloroplasts are often called *phaeoplasts*. Among these algae, the Fucales (*Fucus, Pelvetia, Cystosira*) enclose chlorophyll in all parts of the plant except in the antherozoids. The phaeoplasts appear in all the cells as numerous bodies somewhat analogous to those in the phanerogams. Chlorophyll is, however, formed only in very small quantities in the apical cell and in the oosphere where the spindle-shaped chloroplasts are very small. In the antheridium, the phaeoplasts lose their chlorophyll which is replaced by a crystal of carotin[1] and distribute themselves among the antherozoids in such a way that each of these encloses a single plastid, lacking chlorophyll, but containing instead a carotinoid pigment.

In the Laminariales, the phaeoplasts are encountered in all cells equally and exist in the zoosporangia. Each zoospore contains a typical small phaeoplast possessing a carotin crystal.

The red algae or Rhodophyceae, form in their chloroplasts, in addition to chlorophyll, a red protein pigment, *phycoerythrin*. This is present in such abundance in most species that it masks the chlorophyll. The chloroplasts then are red in color and are called *rhodoplasts*. In the Rhodophyceae, there are grouped together organisms of very different structure: the lower Rhodophyceae, the Bangiales, possess an extremely simple thallus composed of one type of cell. The more evolved Rhodophyceae, the Rhodymeniaceae, the Delesseriaceae, on the contrary, possess a complex vegetative structure with differentiated tissues — meristem, assimilating, and conducting tissue.

In the Bangiales and some Nemalionaceae, such as *Chantransia* and *Rhodochorton*, which are very primitive red algae, the cells are all similar and contain each a rhodoplast of characteristic form (regular disc or star-shaped, ribbon-shaped, etc.), varying with the species and provided with a pyrenoid. The chlorophyll apparatus, therefore, in these algae shows the characteristics of that in the inferior algae (Chlorophyceae). In other Rhodophyceae the shape of the rhodoplasts is closely connected with the various parts of

[1]The term *stigma* or *eyespot* is used for an orange-red body found in the zoospores of most flagellate algae and in the antherozoids of many green or brown algae. The stigma arises sometimes, as is the case for the antherozoids of *Fucus*, from a plastid which loses its chlorophyll and elaborates carotin; but it also often seems to be a differentiated portion of the chloroplast in the case where the cell contains only one large chloroplast or a specialized chloroplast, as in the Euglenas. This stigma plays a photostatic rôle in cells. The carotin seems to render the cell sensitive to light and the cell is oriented in the direction of greatest or least light intensity (positive or negative phototactic response).

the vegetative structure, varying according to the cells in which they are found. They are irregular, or angular, disc-shaped bodies or twisted ribbons. Moreover, chlorophyll is lacking in the rhizoids and sexual organs.

In the Charophytes there is little chlorophyll in the apical cell and small ovoid chloroplasts are often found there containing a large number of starch grains. In this group also, chlorophyll is lacking in the egg and in the antherozoids.

In the bryophytes, the chloroplasts are numerous and similar to those of the phanerogams. Yet in *Anthoceros* there is only one crescent-shaped chloroplast per cell, situated near the nucleus. It is transmitted by division from cell to cell.

It is therefore clearly proved by the study of algae such as the Conjugatae where the chloroplasts are present in all cells, are voluminous, and number only one or two per cell, that these organelles are transmitted from cell to cell beginning with the egg. However, their behavior during fertilization is not yet very clear, for it is very difficult to observe them in the zygote. In the Desmidiales (*Closterium* and *Cosmarium*), in which the cells contain two chloroplasts, KLEBAHN has stated that they fuse two by two in the zygote. Nevertheless, it is difficult to accept this supposed fusion, for it has never been observed in other cases. In another Desmid, *Hyalotheca dissiliens,* in which the cells have only one chloroplast, it seems well established by the recent work of POTTHOFF that one of the two chloroplasts, derived from each of the gametes brought together in the zygote, begins to degenerate and disappears very rapidly. This is likewise reported for other Conjugatae. In *Zygnema,* for example, where there are two chloroplasts in each cell, four are found in the zygote (P. A. DANGEARD and KURSSANOW) and it seems well established by the work of KURSSANOW on *Zygnema stellinum* that the two chloroplasts carried by the male gamete soon degenerate. This is also reported for *Spirogyra crassa* (CHMIELEVSKY), *S. longata* and *S. neglecta* (TRÖNDLE). In the Conjugatae the chloroplasts of the new individual would then have an exclusively maternal origin. In some algae, however, the sequence of events may be otherwise. In a diatom *Rhopalodia,* for instance, each cell encloses a single chloroplast with two pyrenoids. The cells destined to form the gametes divide in their interior to form two gametes each. Now it appears that the single chloroplast divides to furnish each gamete with half a chloroplast possessing a single pyrenoid, because in the zygote the two half-chloroplasts resulting from the union of the gametes fuse to form a single chloroplast with two pyrenoids (KLEBAHN).

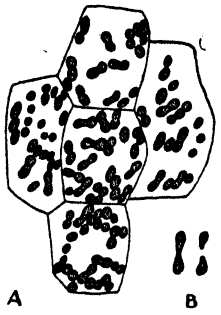


FIG. 13. — *A*, Dividing chloroplasts in leaf cells of *Elodea canadensis*. *B*, Final stages in division of one chloroplast.

The chloroplasts in vascular plants: theory of SCHIMPER:- In vascular plants, pteridophytes and phanerogams, as has been said, chlorophyll is not present in embryonic cells and is formed only in organs exposed to light as the cells of the organs differentiate. Chloroplasts are therefore found only in green tissue. The question as to the origin of chloroplasts remained a long time unsolved. The older botanists concluded that these bodies were formed by differentiation from the cytoplasm or that they were the result of the transformation of starch grains. SCHIMPER (1881-1885) showed for the first time that the chloroplasts are derived in reality from small colorless bodies, protein in nature, which are found in all the colorless tissues of plants. To these bodies he gave the name *leucoplastids*. These were later called *trophoplasts* (MEYER) and *leucoleucites* (VAN TIEGHEM). We shall call them *leucoplasts*[1]. These are very small, rounded, or rod-shaped elements which are found scattered in great numbers in the cytoplasm and which are transmitted from cell to cell by division. All these leucoplasts have the ability to form starch grains, *i.e.*, to condense in the form of starch, the hexoses elaborated in green tissue during photosynthesis and later transported to colorless tissues (roots, tubercles, etc.). Whenever the hexoses accumulate in green tissues in too high concentrations, the leucoplasts become amyloplasts.

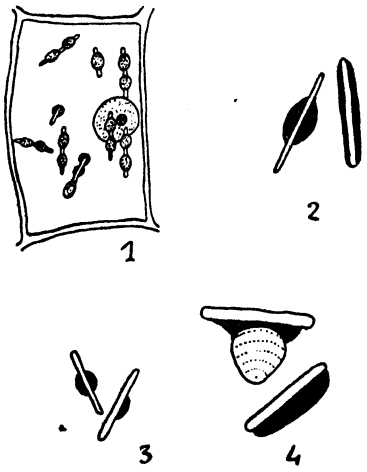


FIG. 14. — Chloroplasts containing protein crystalloids from fruit cells. 1, *Maxillaria triangularis*. 2, 3, *Cerinthe minor*. 4, *Phajus grandifolius*. (After SCHIMPER).

One of the classic examples of this formation of starch through the agency of the leucoplasts is to be found in the root of *Phajus grandifolius* in which SCHIMPER succeeded in following the entire process. In this root, the leucoplasts are indeed rather large, rod- or spindle-shaped, bodies enclosing each along its axis a needle-shaped crystalloid of protein, the product of its elaboration. The starch grain arises in a peripheral region of the thicker part of the leucoplast. Minute at first, this grain grows and very quickly protrudes beyond the plastid which it no longer covers except on one side.

Leucoplasts develop in various ways depending on the organs in which they are found. In leaves, they have only to grow larger

[1]ARTHUR MEYER distinguishes between the *autoplasts* (chloroplasts) and the *trophoplasts*, the latter comprising the *leucoplasts* and the *chromoplasts*. VAN TIEGHEM replaces the term of plastid or plast by that of *leucites* and distinguishes the *leucoleucites* (*leucoplasts*), the *chloroleucites* (chloroplasts), and the *chromoleucites* (chromoplasts). The term plastid having been used by some biologists to mean cell, we replace it therefore by that of plast (which signifies elaborative body) which seems to us much more justified than that of leucite (white body) and we shall distinguish the leucoplasts, amyloplasts, chloroplasts and chromoplasts.

and become green to be transformed into chloroplasts (called chloroplastids by SCHIMPER). In flowers and fruits, SCHIMPER has shown that the carotinoids, xanthophyll and carotin, always form in plastids which he calls *chromoplastids* but which we shall call *chromoplasts*. These may either form directly by transformation of small leucoplasts, which may or may not have previously formed starch, or they may, in other cases, arise by a metamorphosis of chloroplasts whose chlorophyll disappears and is then replaced by a carotinoid. Pigment may appear in the chromoplast either in the amorphous or in the crystalline state. In the former, which is always the case when the pigment is xanthophyll, the chromoplasts affect the same globular or lenticular form as the chloroplasts. In the crystalline state, which is frequently the case when the pigment is carotin, the crystals are contained in variable number in the chromoplasts, and usually take the form of needles

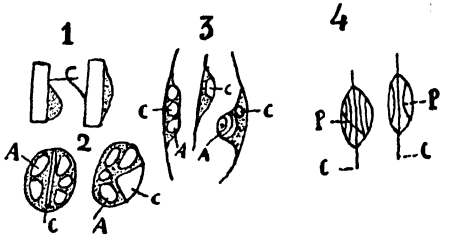


FIG. 15. — Inclusions of the plastids. 1, chloroplasts in epidermal cells of *Hedera* leaves. 2, chloroplasts in palisade parenchyma of leaves of *Achyranthes Verschaffelti*. 3, leucoplasts in young buds of *Canna Warszewickzii*. *A*, starch; *C*, protein crystalloids. 4, chromoplasts in the flower of *Neottia Nidus-avis*. *C*, carotinoid crystal; *P*, protein crystalloid. (After SCHIMPER).

delicately curved in hooks, spirals or slender rods. Sometimes they appear as triangular or rectangular tables, hollow tubes, or spiral ribbons. They are all rhomboidal prisms. The crystals are arranged in parallel or divergent bundles in the chromoplast which, following the contours of the crystal, takes on most varied aspects: rods, spindles, triangles. In some cases, as in the carrot root, the crystals, when once formed, have worn out the greater part of the substance of the chromoplast which made them, and appear either free in the cytoplasm or simply surrounded by a very thin and barely perceptible plastidial envelope. In other cases, there may be formed in chromoplasts not enclosing crystalline pigments, several needle-shaped crystalloids of protein which bring about an elongation of the plastid and give to it the shape of a spindle or rod. It even happens that chloroplasts contain both pigment crystals and protein crystalloids as, for example, in the fruit of *Lonicera xylosteum*, in which the chromoplasts are pear-shaped. The pigment is distributed in the thicker portion of the chromoplast as small needle-shaped crystals, and a protein crystalloid occupies the long axis of the chromoplast and spins out at one of its extremities in the shape of a tenuous appendage. (Figs. 14-17).

All these organelles - leucoplasts, chloroplasts, chromoplasts - belong therefore to the same category of elements to which SCHIMPER gave the general term *plastids* which we shall replace here by that of *plast* (Fr. *plastes*)[1]. They have multiple potentialities, manifestations of which vary with the organ in which the plastids are found. They may remain in the state of leucoplasts and have as their only function the condensation of hexoses into starch, playing the rôle of amyloplasts; or else they may be transformed into chloroplasts or chromoplasts; or from chloroplasts they may be changed into chromoplasts. They all have the ability to elaborate starch and to produce protein crystalloids within themselves in the manner already described for leucoplasts of the root of *Phajus grandifolius* and for the chromoplasts of the fruit of *Lonicera Xylosteum*. Thus chloroplasts of *Cerinthe minor* are traversed by a needle-shaped crystalloid of protein which is prolonged freely at its two extremities.

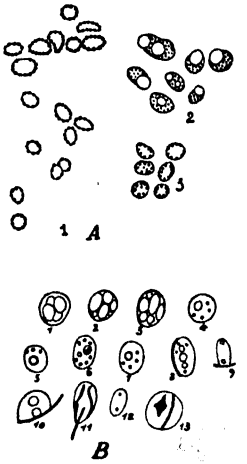


FIG. 16. — Transformations of chloroplasts in mesophyll cells of petals. A, *Lilium tigrinum*. 1, carotin granules at the periphery of green plastids; 2, starch grains in plastids whose chlorophyll has disappeared; 3, completely formed chromoplasts whose starch has been absorbed. B, *Gladiolus* var. 1-3, chloroplasts with large starch grains; 4-7, xanthophyll replaces chlorophyll, starch is absorbed, carotin granules appear; 8-13, carotin crystals appear.

SCHIMPER considers that starch can not arise directly in the cytoplasm but is always the product of plastidial activity. He describes in all details the process of starch formation through the agency of the plastids whether they be leucoplasts or chloroplasts. Let us recall that starch grains grow by apposition and are made up of a dark hilum surrounded by alternately light and dark layers. The hilum is the portion first formed and the alternate layers correspond to zones of different water content which are developed about the hilum during the growth of the starch grains. The hilum may occupy the center of the grain (central hilum) and is then surrounded by regular concentric layers, or it may be situated at one of the poles of the grain whose concentric layers widen and become more and more numerous at the opposite pole (eccentric hilum). There are *simple starch grains, compound starch grains, i.e.*, several grains stuck together, and *half-compound starch grains* which are separate at first but become united by common concentric layers. The difference in type depends upon the method of formation within the plastid. The simple grain with central hilum arises in the middle of the plastid as a small granule which grows, forming regular concentric layers about the hilum, and remains surrounded on all sides by a plastidial wall which grows thinner as the grain enlarges. The simple grain

[1] ***Translator's note.*** **The French words *plastides* and *plastes* are rendered in English by the one word plastid, so that this distinction loses its force in translation. "Plast" is used only in compound words such as "chloroplast", "chromoplast", "leucoplast" etc.**

with eccentric hilum, on the contrary, arises at a point near the periphery of the plastid and, in this case, by enlarging, soon projects beyond the plastid, which no longer covers it except at one of its poles where it takes the form of a cap. The hilum surrounded by the earliest formed layers is then found at the extremity of the grain opposite the plastidial cap. The grain, no longer coming under the influence of the plastid in that region, ceases to grow and enlargement no longer takes place except at the point of contact with the plastid, *i.e.*, at the pole opposite the hilum. There the concentric layers become increasingly numerous and thick. The compound grain arises by a plastid forming several starch grains inside itself, instead of only one. These are in contact with one another and remain small. They are semi-compound grains if they become surrounded by common concentric layers.

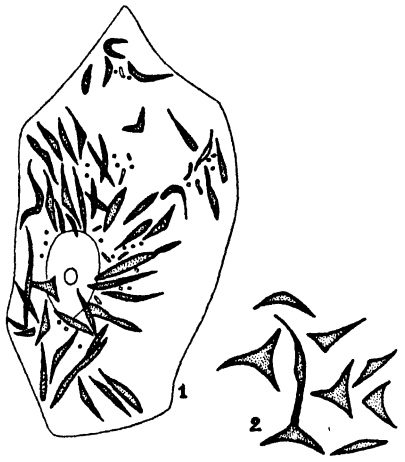


FIG. 17. — Various aspects of chromoplasts derived from chloroplasts in the mesocarp of the fruit of *Rosa canina* (*in vivo*).

On the basis of all his investigations, as well as those of SCHMITZ on the algae, SCHIMPER was led to consider the plastids as component parts of the cell, incapable of arising *de novo*, being transmitted by division from cell to cell beginning with the egg, so that plastids of higher plants, according to him, are comparable to the chloroplasts which are encountered permanently in many algae, but with this difference: in the algae the plastids always keep their chlorophyll and remain as green plastids, while in higher plants they appear first as leucoplasts and do not become chloroplasts except in stem and leaf tissue.

ARTHUR MEYER (1883) working at the same time, confirmed the theory of SCHIMPER which was verified also as far as the chromoplasts are concerned by COURCHET (1888).

It is fitting to call attention to the observation that the plastids, at the same time that they are elaborating starch and pigments, are capable of producing inside themselves small refracting granules which reduce osmic acid (Fig. 18). To these, which are sometimes very numerous, a lipide constitution has been attributed. These granules were described long ago by NÄGELI, GODLEWSKI, SCHIMPER, MEYER. They have been the object of more recent research by MEYER who has opposed the idea of their lipide nature and considers them to be composed of α-β hexylenealdehyde, a waste product of photosynthetic assimilation found in the products of the distillation of the leaves. MEYER calls these formations elaborated by the plastids *Autoplastensekret*. We shall see, nevertheless, that this interpretation has not been confirmed

and that these granules present the histochemical characteristics of lipides and not those of aldehydes (*Cf.* p. 209).

Just what these granules signify is still very obscure. In cells in which the plastids never form starch, the granules are often considered as assimilation products replacing starch. It has been noticed, sometimes, that these granules appear in large numbers during the period preceding the formation of starch and of chlorophyll and carotinoid pigments, only to disappear as soon as these products have been formed. It was therefore thought that they might be an intermediate product contributing to the formation of starch or pigment. It has also been shown that very frequently similar granules appear in great numbers in plastids as they degenerate in the cells of flowers which are beginning to take form. In this case, the presence of the granules can only be explained as a breaking down of the plastidial lipoprotein complex, *i.e.*, as a process called *lipophanerosis* (demasking of lipides. *Cf.* p. 203, 205).

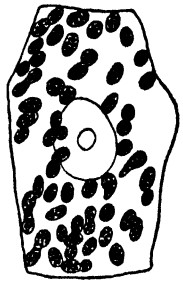


Fig. 18. — Epidermal cell in a petal of *Iris belgica* with lipide granules in the chloroplasts.

Finally, large globules have recently been described in the plastids of various Cactaceae (*Cephalocereus, Echinocereus,* etc.). They present the histochemical characteristics of phytosterol and form in the plastid exactly as do starch grains. These globules always precede starch formation and disappear the moment that starch appears. It was therefore supposed by those who did the work that these globules of phytosterol constituted a material which served in the building up of starch (Savelli, Miss Manuel) (Fig. 19).

For a very long time it was impossible to obtain paraffin sections of preserved and stained plastids, at least of leucoplasts, for they were destroyed by all fixatives then used, and only the much more resistant chloroplasts were obtained. Schimper's and Meyer's observations were therefore made exclusively from very well executed studies of living material. These observations were necessarily incomplete for it is absolutely impossible to distinguish plastids in living embryonic cells and until the present writing they have been seen only with the very greatest difficulty in colorless differentiated tissue, such as the root, and then only in favorable cases. The classical observations of Schimper on the method of starch formation were based for the most part on examples particularly favorable for study, such as the root of *Phajus grandifolius,* in which the leucoplasts are very massive. Therefore the theory of Schimper and Meyer was very largely hypothetical and was based chiefly on what seemed to them likely to be true, and on what can be observed in many algae in which the chloroplasts behave like component parts of the cell.

Numerous workers contested this theory of starch formation, among others, Belzung, who did not succeed in distinguishing

leucoplasts in living cells and asserted that starch arises most often within the cytoplasm itself and that chloroplasts form by cytoplasmic differentiation. The development of the plastids was very imperfectly known and even the most characteristic forms which the plastids take on were not known. Progress in this matter was not marked until much later, from 1910 on, when research was carried out with so-called mitochondrial technique which makes possible the preservation of unaltered plastids, clearly stained on paraffin sections. This will be taken up later. These methods have made it possible to follow with the greatest precision the entire life history of the plastids during cellular development; to bring out different aspects which up to then had not been perceived; and to make important discoveries which have confirmed the ideas of SCHIMPER and MEYER by completing them and giving them precision.

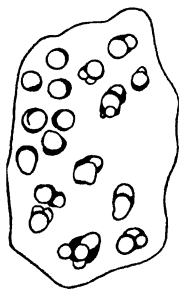


FIG. 19. — Chloroplasts in a parenchymatous cell of *Echinocereus procumbens* showing large inclusions of phytosterol. (After Miss MANUEL).

Chemical nature and structure of plastids:- Plastids for a long time were thought to be exclusively protein in nature. It was however believed, without anyone being able to furnish proof for it, that plastids must enclose a lipide substance, probably lecithin, in which the chlorophyll was supposed to be dissolved. GAUTIER, HOPPE-SEYLER and STOKLASA even formulated the hypothesis that chlorophyll is a chlorolecithin. The presence of lipides in chloroplasts is not questioned today and MENKE, chiefly, seems to have furnished a proof of it. GRANICK, by triturating and then centrifuging tobacco and tomato leaves, was able to isolate a certain quantity of chloroplasts and to obtain their microchemical analysis. GRANICK thus managed to separate proteins from lipides. Recent work, which will be discussed later, has shown by histochemical reactions that in reality all plastids, like the cytoplasm, are composed of lipoproteins in which, however, the lipides (compound lipides, probably lecithins) are much richer than in the cytoplasm and give to the plastids their color characteristics.

The importance of the plastids in photosynthetic assimilation has led authors for a long time to attribute a structure to these organelles. The leucoplasts appear homogeneous and the chloroplasts, and chromoplasts especially, have been studied from this point of view.

Let us recall that these organelles are formed of a lipoprotein substratum on which the chlorophyll is fixed (chlorophyll *a* and *b*), accompanied by a small quantity of carotinoid pigments (carotin and xanthophyll).

PRINGSHEIM, TSCHIRCH, and CHODAT held that the chloroplasts consist of a spongy material impregnated with a fluid substance of an oily consistency (lipochlorine of PRINGSHEIM) serving as a sub-

stratum for the pigments. SCHMITZ attributed a fibrillar structure to algal chloroplasts and SCHWARZ considered the chloroplasts of higher plants to be composed of two substances, the one, chloroplastin, formed of basic filaments, lying closely together, containing chlorophyll grains, and the other a colorless substance, metaxin, interposed among the filaments.

SCHIMPER and MEYER held that chlorophyll in chloroplasts was found as inclusions, the *grana,* so small as to border on the limits of visibility. These grana they found to be very numerous and difficult to distinguish, giving the impression that the pigment is found in a diffuse state in the plastidial substratum. According to these two investigators, xanthophyll is distributed in the same way in chromoplasts and only carotin, when it is not in a crystalline state, exists in the form of clearly differentiated grana.

Various arguments of a theoretical nature next led physiologists to think that the chloroplasts must have a homogeneous structure. A comparative study of the spectra of a chlorophyll solution and of a living green leaf show, indeed, that the absorption bands do not occupy the same positions in the two cases, and that those for the living leaf coincide with those shown for a colloidal solution of chlorophyll. It is known that solutions of chlorophyll break down rapidly in light in the presence of oxygen. There is then an oxidation of chlorophyll. In the chloroplast, on the contrary, the chlorophyll manifests a great stability which could only be shown by a colloidal solution of chlorophyll united to other colorless colloids. Certain facts, furthermore, lead to the supposition that chlorophyll may be combined in the plastidial substratum either with proteins or with lipides. Hence it has been thought that chlorophyll must be uniformly distributed throughout the entire mass of the chloroplast and that the latter is constituted of a hydrogel formed of various colloids, some colorless, others colored (SIEBOLD), or formed of colloids containing chlorophyll chemically combined with colorless colloids. According to PONOMAREW, LEPESCHKIN, and KÜSTER, the gel of the chloroplast is in a semi-fluid state and the variations of form undergone by this organelle may be explained by a too weak tension between the cytoplasm and the chloroplast. Investigations of PRICE, SCARTH, LAPICQUE, GUILLIERMOND and MANGENOT have shown, moreover, that chloroplasts, although sometimes giving the impression in direct light of having structure, are always optically empty under the ultramicroscope and are not seen except for their color and their faintly luminous contours. This confirms the opinion stated above. Finally, mitochondrial technique, which best conserves the chloroplasts and makes their lipides insoluble, always reveals these organelles as absolutely homogeneous, while those techniques which dissolve lipides bring out a heterogeneous structure which may be supposed to correspond to an alteration.

More recent cytological work, however, would tend to prove, on the contrary, that the chloroplasts do have a structure. According to ZIRKLE, they are formed of a chlorophyll-containing hydrogel

pierced by colorless pores, encircling a central vacuole which, when visible, contains starch. The plastid is coated by an adsorbed layer of cytoplasm. ZIRKLE thinks starch is formed in the vacuole of these chloroplasts. Living chloroplasts, however, are very delicate organelles and one runs the risk of some alteration taking place during observation, so that it is wise to be very cautious in the acceptance of this structure which has never received any confirmation.

More interesting is the later work of numerous authors whose results are in agreement and will be briefly analyzed here. In the first place the works of MENKE, KÜSTER, HEITZ, and FRIEDL WEBER have shown that chloroplasts are clearly birefringent. This is particularly easy to demonstrate in the chloroplasts of the Conjugatae (*Mougeotia, Zygnema, Closterium, Spirogyra*). In polarized light the chloroplasts are luminous and show a green polarization color. MENKE found that in the chloroplasts of the Diatomaceae, the polarization colors vary from reddish brown to red. According to WEBER, reddish brown to red polarization colors are not peculiar to the chloroplasts of the algae (diatoms, *Spirogyra, Zygnema, Vaucheria*) but are also found, together with green polarization colors, in the chloroplasts of higher plants (*Polygonatum officinale, Bellis perennis, Elodea canadensis*). WEBER finds that the perception of these colors depends upon the intensity of the light in which the plants are observed. Furthermore, the investigations of MENKE, DOUTRELIGNE, WAKKER, WIELER, HEITZ, WEBER, HUBERT, GEITLER, DESCHENDORFER, BEAUVERIE, WEIER and STRUGGER seem to confirm in chloroplasts the existence of a structure, described by SCHIMPER and MEYER fifty years ago, according to which chlorophyll is fixed on grana suspended in a colorless stroma[1]. These grana according to WIELER are enclosed within a peripheral layer of the stroma. HEITZ thinks the grana are small bodies, measuring from 0.3-1.7μ in diameter, and appearing flat and discoid in shape, which explains the striated structure often visible in chloroplasts when observed in certain positions. DOUTRELIGNE describes them as rods or granules which, according to the conditions present, may assemble like strings of beads, may separate, or may become confluent. BEAUVERIE confirms these data without admitting, however, that the structure is general, for he thinks that in certain plants the chloroplasts may be homogeneous.

The chemical nature of the grana is still unknown. WIELER concludes that they are formed of an essential oil in which the chlorophyll is dissolved. HEITZ considers them simply as grains

[1]Some even older observations would seem to support this view. CHODAT, for instance, in the pseudobulb of *Calanthe Sieboldi*, described chloroplasts which are round at first but capable of changing to a dumb-bell shape by elongation and stretching at their middle region. Now the two swellings remain filled with chlorophyll while the slender part connecting them becomes colorless. In the mesophyll of leaves and bracts of *Iris germanica* as well, we have seen large chloroplasts showing at one extremity, or both, a sort of slender, colorless appendage. EMBERGER observed that in the bulb scales of *Lilium candidum* the chloroplasts are surrounded by a colorless layer which seems to indicate that chlorophyll, like the starch grain, has been laid down within the plastid.

of chlorophyll. Still others think they are lipides holding the pigment in solution (MENKE, WEBER).

With the study of this structure the question of the double refraction of chloroplasts has been brought up again. While KÜSTER concludes that double refraction is due to the ground substance of the chloroplast, MENKE, HEITZ, and WEBER attribute it to the grana which, accordingly, they believe correspond to doubly refractive lipide inclusions. MENKE and WEBER observed that after a prolonged stay in water, the chloroplasts are the seat of the production of green myelin filaments which correspond to the fibrils of SCHWARZ and which arise from the grana[1]. Now WEBER showed that these myelin figures when seen in polarized light present the same double refraction and same red color as do the grana, which he thinks tends to prove that in the chloroplasts only the grana are doubly refractive and that these elements correspond to liquid cristals[2].

It is known, however, that GIROUD had recorded that the chondriosomes of animal cells, which we will see later on are akin to plastids, appear doubly refractive. WEBER to confirm his opinion examined chondriosomes and leucoplasts of various plants in polarized light but was not able with certainty to prove that they are doubly refractive. It is known, of course, that carotin in chromoplasts, when it is not crystallized, appears as clearly separated granules in the colorless substance of the plastid. Now in examining such chromoplasts in polarized light, WEBER was able to demonstrate that the double refraction is localized exclusively at the level of the pigment granules. This seems to demonstrate that the substratum of the plastids is not doubly refractive and that this characteristic is due to the grana in chromoplasts and chloroplasts alike. The problem of the structure of the chloroplasts is certainly very complicated, since it is difficult to avoid alterations in living material during observation, but the tendencies are manifestly in favor of a heterogeneous structure. Most authors (HEITZ, DOUTRELIGNE, WIELER, DESCHENDORFER, WEBER, PEKAREK, GEITLER) agree that the chloroplasts are composed of small lipide discs containing chlorophyll and embedded in a hydrophilic stroma. WEBER thinks these discs are responsible for the fluorescence of chlorophyll and the double refraction of the chloroplasts.

Recently FREY-WYSSLING has attributed a lamellate submicroscopic structure to these discs, consisting of a series of parallel layers of protein, lecithin, chlorophyll and carotinoid pigment. The scheme of this author implies that the phytol groups of the chlorophyll molecules are interposed between aliphatic chains of the lecithins, the porphin residues being arranged in a monomolecular

[1]By placing *Spirogyra* in a 1-2% solution of sodium oleate, WEBER saw the spiral bands of the chloroplast swell and form protuberances on their surfaces reminiscent of myelin figures and sphaerocrystals. These formations are green like the chloroplasts and correspond to the lipide phase of the chloroplast.

[2]WEBER thinks these grana may correspond to the granules considered by MEYER to be formed of α-β hexylene-aldehyde, a product of photosynthesis, which we will speak of again farther on.

layer. This supposed submicroscopic structure makes it possible to form new hypotheses for the mechanism of chlorophyll assimilation (BAAS BECKING and HANSON, 1937).

As for the pyrenoids which are found in the chloroplasts of many algae, they are still variously considered and opinions as to their significance are not yet well fixed. SCHMITZ, CHMIELEVSKY, and LUTMAN consider them permanent organelles, multiplying by division. SCHIMPER, KLEBS and STRASBURGER saw them disappear and maintain that they are bodies which form *de novo*, an opinion which today seems demonstrated. It was thought that the pyrenoids constituted a reserve protein elaborated by the plastids. Other authors, on the contrary, think they play an important rôle in the formation of starch which always arises in their interior (LUTMAN, MCALLISTER) or in contact with them (CHADEFAUD).

In terminating this discussion as to the structure and chemical nature of chloroplasts, attention is called to the ability of chloroplasts to reduce silver salts, especially in a 1% solution of silver

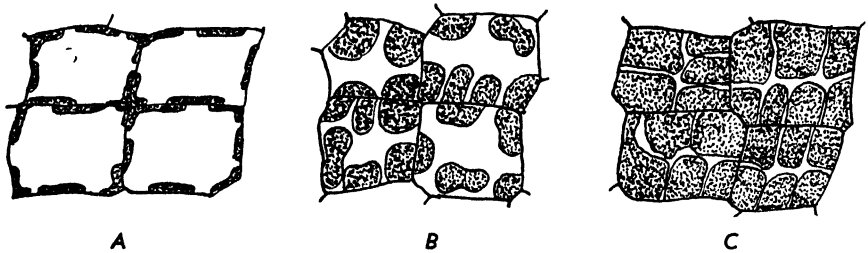


FIG. 20. — Positions taken by chloroplasts in cells of seedlings of *Saccorhiza bulbosa* in intense (A) and weak (C) illumination. B, intermediate position. (After SAUVAGEAU).

nitrate. This property, discovered by MOLISCH and called the MOLISCH reaction, is connected neither with chlorophyll nor the carotinoid pigments, is produced only in living tissues and seemed to MOLISCH to be independent of light. MOLISCH attributed it to the presence of formaldehyde in the chloroplasts. Investigations of GAUTHERET have led to important information about the conditions under which the MOLISCH reaction is carried out. This research showed that light plays a rôle in the reduction of silver nitrate by chloroplasts: the reduction begun by the action of light may continue subsequently in the dark. GAUTHERET's work proved finally that this reaction is not due to the presence of formaldehyde in the chloroplast but to reducing substances still unknown.

More recently GIROUD and his collaborators claimed that the Molisch reaction is to be attributed to the presence of ascorbic acid within the chloroplasts which, therefore, these workers believe to be the source of this substance.

Movement of chloroplasts:- It has been known for some time that chloroplasts are capable of moving from place to place and, depending upon the intensity of the light, are capable of placing themselves on one side or the other of a cell. But for a long time it has been said that they play only a passive rôle in these move-

ments, which those who hold this opinion think are caused exclusively by cytoplasmic currents. From observations of WEISS, SCHIMPER, and KÜSTER it appears that chloroplasts are capable of becoming distorted and of showing amoeboid movements. This is also recorded for leucoplasts, as will be seen later. Now SAUVAGEAU showed by observations of seedlings of *Saccorhiza bulbosa* that when exposed to intense light the chloroplasts, during their movements, present contractions and dilations which can be explained only by movements which they make themselves. SENN, who recognizes the capacity of chloroplasts to move themselves, attributes this to the presence of a cytoplasmic sheath surrounding them which he calls a *peristromium.* This, according to SENN, gives rise to pseudopodia which permit the chloroplasts to change shape and place. The existence of this peristromium, however, has never been confirmed and remains very problematical.

Chapter VII

THE CHONDRIOME

General conceptions. What is meant by chondriome in animal cells:- As the chondriome was observed first in animal cells, it seems necessary before beginning its study in plant cells, to recall as briefly as possible what is understood under that heading in animal cytology.

The observations of ALTMANN cited in the previous chapter, although exact, did not at first hold the interest of cytologists and the bioplasts described by him were for a very long time confused with the granules of ARNOLD brought out in most animal cells in the time that followed by means of vital stains. It is known now that these granules correspond to vacuoles and, in consequence, have a quite different significance. It was believed that these granules were merely artifacts and it was not until very much later that the work of BENDA, MEVES, REGAUD and FAURÉ-FREMIET demonstrated, by the use of special methods similar to those of ALTMANN, the constant presence of small organelles in the cytoplasm of animal cells. (Figs. 21, 22). They were somewhat similar in shape and dimension to bacteria and were afterwards identified with the bioplasts of ALTMANN and the fila of FLEMMING. These elements, whose width does not exceed 0.5-1μ, were named *chondriosomes* by BENDA (1906) and *plastosomes* by MEVES. The general term *mitochondria* is often applied to them. They appear now as granules called *mitochondria* (BENDA), now as rods or long, undulating, sometimes branched filaments called *chondrioconts*. One of these shapes may change into the other. The granule is capable of elongating into a rod, then into a filament and this latter may, in turn, fragment into granules. Mitochondria assembled in small chains were called *chondriomites* by MEVES. It was later recognized that this very rare shape sometimes represents a transition form resulting from a fragmentation of chondrioconts but more often still, corresponds merely to an alteration of the chondrioconts caused by the fixatives (LEVI). MEVES proposed the term *chondriome* (1908) or *plastome* (1910) for the entire chondriosomal content of a single cell.

The chondriosomes are made up of lipoproteins, very rich in lipides (phosphoaminolipides), and can not be brought into evidence except by the use of special fixation techniques, called mitochondrial, which do not affect the phosphoaminolipides. Fixatives containing alcohol or acetic acid, *i.e.*, those which were most currently used before the discovery of chondriosomes, do not reveal them. This explains why, although visible in living material, they were able to pass unperceived for so long. Mitochondrial techniques consist in fixing cells with a mixture of chromic and osmic

acids (method of BENDA and MEVES) or with a mixture of formol and potassium bichromate (method of REGAUD), and then in following the fixation with a more or less prolonged treatment of a 3% solution of potassium bichromate, an operation called post-chromatization which renders the chondriosomal lipides insoluble. Once fixed, the chondriosomes stain clearly with iron haematoxylin, acid fuchsin and crystal violet. They appear in the homogeneous, barely-stained cytoplasm as intensely stained elements, with a very clear outline and they very much resemble bacteria. It has been shown that chondriosomes are made up of a lipoprotein complex and that their affinity for stains is due to the lipides which they enclose. Fixatives containing alcohol or acetic acid destroy these lipides and the chondriosomes lose their chromaticity.

In young cells, mitochondria generally predominate among the chondriosomes. At a later stage they elongate into chondrioconts which is the most usual form found in mature cells. The chondriosomes are permanent formations and many cytologists consider that they are incapable of forming *de novo* and increase in number only by division of pre-existing chondriosomes. At first the chondriosomes were regarded as organelles in whose interior were formed most of the products elaborated in the cell (fat, zymogen, pigments) and whose rôle was the same as that of the plastids in chlorophyll-bearing plants. But observation of living material using tissue-culture technique does not confirm this idea. The very accurate observations of NOËL on the liver of rats are the only ones made so far which seem favorable to this belief. NOËL has shown that when an exclusively nitrogenous diet is given the rats, the chondriosomes of their liver cells, which are normally in the state of chondrioconts, become large, round bodies filled with protein. It seems, therefore, that the chondriosomes accumulate protein and act as proteoplasts. The phenomenon is reversible and, if the nitrogenous food is suppressed, the proteoplasts lose their protein and resume the form of chondrioconts. The rôle of the chondriosomes is still very obscure in spite of this single observation.

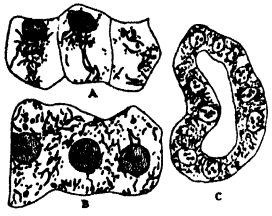


FIG. 21. — The chondriome. A, frog's liver. B, salamander's liver. C, frog's kidney. Regaud's method.

The chondriome in plant cells:- Chondriosomes in plant cells were discovered even as early as 1904 by MEVES who found them in the nurse cells of pollen of the Nymphaeaceae. His results were subsequently confirmed in various organs of the phanerogams by the research of a certain number of investigators (SMIRNOW, NICOLOSI-RONCATI, BONAVENTURA), among whom DUESBERG and HOVEN will be given a special place, for in 1910 they produced excellent figures of the chondriome in embryonic cells of the pea and bean.

Beginning with the year 1910, the aspect of the question changed and the almost simultaneous work of PENSA (1910), LEWITSKY (1911), and our own work (1911) showed a relationship between chondriosomes and chloroplasts. But, as will be seen, from that moment on, investigators found themselves face to face with a problem which it took several years of patient and laborious research to solve.

A study of chondriosomes in the different plant groups has demonstrated that these elements exist in every cell except, however, among the bacteria, where it has not yet been possible to reveal them, and in the Cyanophyceae in whose cells it is at present demonstrated that they are not found.

The chondriome in fungi:- The study of the chondriosomes is relatively simple in plants lacking in chlorophyll, *i.e.*, the fungi, where we described them for the first time in the ascus of *Pustularia vesiculosa* (1911). Chondriosomes were, after that, cited in the most varied fungal groups: Myxomycetes (VONWILLER, COWDRY, LEWITSKY, MANGENOT), Plasmodiophoraceae (MILOVIDOV), Chytridiaceae (POISSON and MANGENOT), Blastocladiaceae (WINSLOW HATCH), Saprolegniaceae (RUDOLPH, GUILLIERMOND), Peronosporaceae (LEWITSKY, H. EDSON, DUFRÉNOY, SAKSENA, Miss SYNGALOWSKY), Dermatophytes (GRIGORAKI, NEGRONI), Mucoraceae (GUILLIERMOND, MOREAU), Hemiascomycetes (GUILLIERMOND, VARITCHAK), lower Ascomycetes: *Endomyces Magnusii, Endomyces fibuliger* (GUILLIERMOND), yeasts (JANSSENS and VAN DE PUTTE, GUILLIERMOND, HENNEBERG, NEGRONI, TREDICI, VERONA), higher Ascomycetes: Pezizales, *Penicillium glaucum* (GUILLIERMOND, JANNSENS and HELSMORTEL), Ustilaginaceae and Uredinaceae (M. and Mme. MOREAU, BEAUVERIE), *Autobasidiomycetes* (GUILLIERMOND, BEAUVERIE, SARAZIN, Miss DUCHAUSSOY).

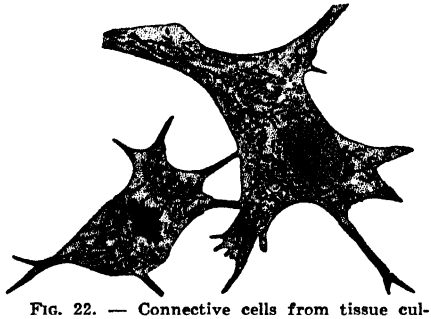


FIG. 22. — Connective cells from tissue cultures of guinea pig prepared by the mitochondrial method. (After MAXIMOV).

In almost all fungi, the chondriosomes predominate in the form of chondrioconts, generally very elongated, sometimes branched, and orientated in a direction parallel to the longitudinal axis of the hyphae. In the Myxomycetes and Plasmodiophoraceae, however, there are present only mitochondria or short rods.

Development of the chondriome:- The chondriosomes are found at all times in the cytoplasm of fungi. They are distributed among the spores in the sporangia and asci and are likewise found in the conidia (*Penicillium glaucum*) and in the buds of yeasts (Fig. 23).

In the Myxomycetes and Plasmodiophoraceae in which COWDRY, LEWITSKY, and MILOVIDOV have studied the chondriosomes in all stages of development (spores, zoospores, myxamoebae, plasmodia), these elements remain constantly in the state of mitochondria or short rods and never become chondrioconts. During sporogenesis they are distributed among the spores (Fig. 24).

The development of the chondriome in one of the Blastocladiaceae, *Allomyces arbusculus*, is known from a recent study of WINSLOW HATCH. In the mycelium the chondriome is represented exclusively by long slender chondrioconts which appear to thicken at the extremities of the hyphae. At the time of gametogenesis, walls at the extremities of the hyphae cut off two gametangia, the female being terminal, the male subterminal, both enclosing numerous chondrioconts which are more abundant in the female than in the male gametangium. The chondriosomes are distributed about the various nuclei of the two gametangia, forming around each nucleus an entangled network of chondrioconts. Then, at the close of gametogenesis, the chondrioconts in each gamete undergo a fragmentation by which they are reduced to numerous, very small, mitochondria. These subsequently grow, then fuse, forming about each nucleus a sort of reticulate mantle which seems to be transformed later into a chromatic, homogeneous cap appressed to the nucleus on one side (nuclear cap). Each gamete when mature encloses, therefore, a nuclear cap seemingly of mitochondrial origin, occupying the regions of the cell opposite to the insertion point of the flagellum. HATCH compares this cap to the limosphere of moss antherozoids and to the "Nebenkern" of some animal spermatozoids (Diptera). Nevertheless the mitochondrial origin of this nuclear cap seems still to demand some verification (Fig. 25).

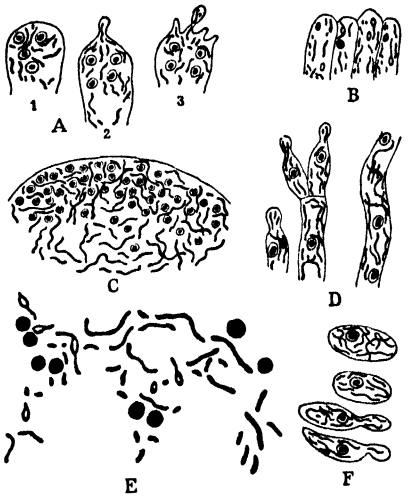


FIG. 23. — The chondriome in fungi. A, developing basidium of *Coprinus;* B, young basidia of *Psalliota campestris;* C, young sporangium of *Rhizopus nigricans;* D, conidiophore of *Penicillium glaucum;* E, tissue from the foot of *Psalliota campestris;* F, yeast, *Sporobolomyces roseus.*

The development of the chondriosomes is known particularly in the Saprolegniaceae in which we have been able to follow different species (*Saprolegnia, Achlya, Leptomitus*) with the greatest accuracy during their entire development, not including the sexual process (Figs. 26, 27). The chondriosomes of these fungi appear in the extremities of growing hyphae as relatively large mitochondria. Immediately behind the tip, these elements begin to elongate, first becoming rods, then thin, undulating and often branched, chondrio-

conts. The chondrioconts become thinner and thinner as they elongate which seems to indicate that they are formed by a progressive stretching of the mitochondria. In the young zoosporangia, the chondriosomes are all in the state of mitochondria which subsequently, together with the cytoplasm, assemble about each of the numerous nuclei, thus outlining the circumferences of the future zoospores which are seen as separated by hyaline areas. All the zoospores when mature enclose a chondriome made up exclusively of mitochondria which, at the time of germination, are transformed in the germinating tube into chondrioconts.

The development of the chondriosomes is also very well known

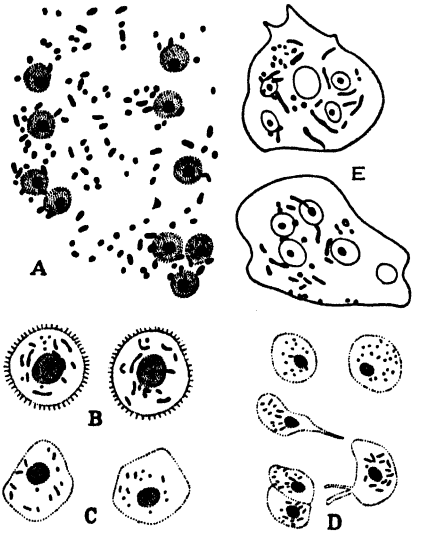


FIG. 24 (left). — The chondriome in Myxomycetes and the Plasmodiophoraceae. A, fragment of the plasmodium of *Physarum* (original); B, spores of *Fuligo septica* (after LEWITSKY); C, spores of *Hemitrichia vesparum* (after COWDRY); D, spores and zoospores of *Fuligo septica* (after VONWILLER); E, young plasmodia of *Plasmodiophora Brassicae* (after MILOVIDOV).

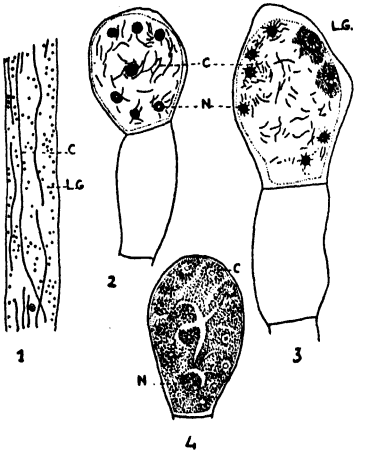


FIG. 25 (right). — *Allomyces arbusculus*. 1, elongated chondrioconts in the vegetative filament. 2, 3, the chondrioconts grouped about the nuclei in the gametangium. 4, mitochondria surrounding the spores as they are cut out. *C*, chondrioconts; *L.G.*, lipide granules colored brown with osmic acid; *N*, nucleus. Champy-Kull method. (After HATCH).

in the ascus of *Pustularia vesiculosa*. It was here that they were studied for the first time in the fungi by us and then by JANSSENS and HELSMORTEL. They present a series of interesting phenomena. All of the ascogenous hyphae, those from which the asci will be formed, show a chondriome made up of numerous chondrioconts densely clustered about each nucleus. It is known that the ascus forms at the terminal portion of these hyphae. This terminal, recurved, crosier-like portion is occupied by a binucleate cell whose two nuclei divide simultaneously. Then two transverse walls form, marking off three cells, of which the middle one, that occupying the arch of the crosier, encloses two nuclei whereas the others, that at the extreme tip and that at the base, contain only a single nucleus. The middle binucleate cell destined to form the ascus, contains at first, around each of its two nuclei, a small mass made up of the

numerous, densely clustered, chondrioconts. Then the cell undergoes a nuclear fusion and when that is completed, the two chondriosomal masses fuse around the single nucleus. After nuclear fusion, the ascus enlarges and grows longer progressively to form an elongated voluminous cell. During this process, the nucleus maintains a somewhat central position while the chondrioconts, which have been grouped about the nucleus, spread out through the entire cytoplasm which in this phase is filled with small vacuoles. At the same time, at one or several points on their long axis, the chondrioconts usually form small swellings, each occupied by a vesicle (Fig. 28).

When the growth of the ascus is complete, but a little before the first mitosis occurs, these vesicles disappear and the chondrioconts, all oriented in the direction of the longitudinal axis of the cell, manifest a tendency to elongate. At this stage a cytoplasm (sporoplasm), dense and rich in chondrioconts, differentiates in the central region of the ascus while the basal and apical portions destined to make up the epiplasm, remain filled with small vacuoles. The nucleus which occupies the center of the sporoplasm then undergoes three successive mitoses during which, it is seen, the chondrioconts remain scattered in the sporoplasm except in the region occupied by the asters where they are completely absent. The divisions completed, each of the resulting eight nuclei remains connected with its aster by a small protuberance at the end of which the centrosome still persists. It is not long before the astral fibres themselves recurve so that, in section, each nucleus then appears as if surmounted by a parasol on whose surface there will first form the limiting membrane of the future ascospore. Now the whole region of the future spore occupied by the centrosome and aster shows no chondriosome at all. All these elements are found exclusively at the opposite pole, where they form a compact mass of entangled chondrioconts. The ascospores then enlarge and become surrounded by a cellulose wall. Not until then do the centrosome and aster disappear. At the same time the nucleus becomes centrally placed in the ascospore and the chondriosomes are distributed throughout the cytoplasm. Only a few chondriosomes remain in the epiplasm.

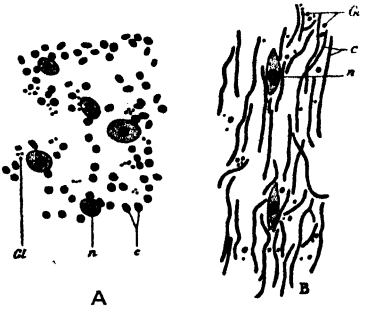


Fig. 26. — Fragments of protoplasm of *Achlya*. A, at the extremity of a growing filament; B, an older filament. *c*, chondriosomes. *Gl*, lipide granules blackened by osmic acid. *n*, nucleus. Mèves' method, stained with acid fuchsin.

In the ascus of one of the Hemiascomycetes, *Ascoidea rubescens*, VARITCHAK has described in more recent work, a vesiculation of chondriosomes analogous to that recorded for the higher Ascomycetes.

It has also been possible to follow the life history of the chondriosomes during the development of some of the Agaricaceae, in

particular that of *Agaricus campestris* (BEAUVERIE, GUILLIERMOND, SARAZIN) and in *Coprinus fimetarius* (Miss DUCHAUSSOY). All the hyphae which compose the plectenchyma of the foot and of the cap of the sporophore have a chondriome almost exclusively made up of long chondrioconts. In young basidia, before and after nuclear fusion, there is also found a large number of chondrioconts lying parallel to the long axis of the basidium. As in the case of the asci, the chondrioconts frequently show small vesiculate swellings on their long axes. When the two nuclear divisions of the basidium are completed, the chondrioconts fragment into short rods and migrate with the cytoplasm and nuclei to the basidiospores, each of which encloses a chondriome formed of numerous rods. In the course of germination, these rods move into the germinating tube where they multiply and elongate and become long chondrioconts in the primary mycelium.

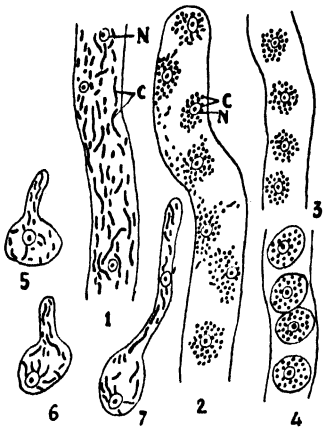


FIG. 27 (left). — Development of the chondriome in *Leptomitus*. 1, chondrioconts in the vegetative filament; 2, 3, fragmentation of the chondrioconts and grouping about the nuclei during the formation of the zoospores; 4, granular chondriosomes in the zoospores; 5-7, germination of the zoospores, elongation of chondriosomes into chondrioconts. *C*, chondriocont. *N*, nucleus. Meves' method, stained with acid fuchsin.

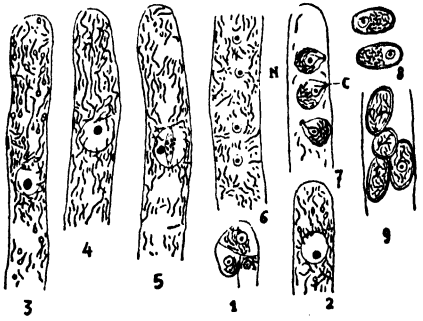


FIG. 28 (right). — Development of the chondriome in the ascus of *Pustularia vesiculosa*. 1, very young ascus after nuclear division; 2-4, vesiculation of the chondriosomes during growth of the ascus. 5, first mitosis; 6, fragment showing clear zone about each nucleus corresponding to the aster; 7, formation of the ascospores; 8, young ascospores; 9, older ascospores. *C*, centrosome; *N*, nucleus. Meves' method.

We have been able to observe the chondriome in some living fungi: *Endomyces Magnusii, Saccharomycodes Ludwigii* but the Saprolegniaceae are particularly favorable for this type of study, as we have shown in our research. MEYER as early as 1904 observed living chondriosomes in *Achlya* and described them as leucoplasts. We have since been able to follow with very great clearness the entire development of the chondriome from the germination of the zoospores to the formation of the zoosporangia in several living fungi of this group: *Saprolegnia, Achlya, Leptomitus*. The sexual cycle has not been studied as sexual reproduction is not easy to obtain in culture (GUILLIERMOND).

This study has confirmed results obtained with mitochondrial technique and has thus very emphatically shown that the chondrio-

somes are permanent elements which are found in all parts of the fungi and which are never seen to arise *de novo*, even in prolonged examination of living material. In addition, the presence of numerous division figures of chondriosomes and the regular distribution of these elements among the zoospores seem to indicate clearly that the chondriosomes are transmitted by division from cell to cell and never arise *de novo*. Proof of this, however, is difficult to furnish, but we shall see later that certain indirect arguments drawn from the study of plastids in chlorophyll-bearing plants seem favorable to the opinion that chondriosomes maintain their individuality in the course of development.

Research on the development of chondriosomes, carried out by direct observation as well as by mitochondrial techniques, has not furnished any information as to the rôle of these elements and has not brought any concrete facts to bear on their participation in the secretory phenomena so often attributed to them in animal cytology.

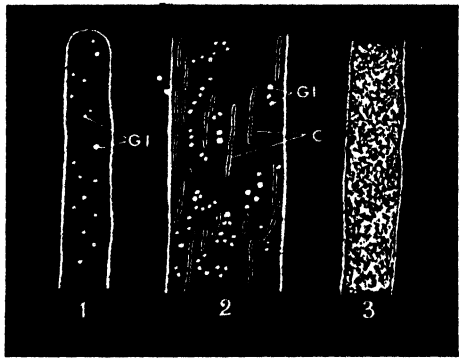


FIG. 29. — Portions of filaments of *Saprolegnia* observed with the ultramicroscope showing (1) only the lipide granules (*Gl*) illuminated, (2) the chondriosomes (C) also and (3) the coagulated protoplasm.

It is true, as has been seen, that in the asci of *Pustularia vesiculosa* and of *Ascoidea rubescens* and in the basidia of the Agaricaceae the chondrioconts form on their long axes vesicular swellings which have the same appearance as those brought about by the formation of starch grains in the leucoplasts of the phanerogams. This has also been observed by LEWITSKY during the formation of the oogonium in the Peronosporaceae and more recently by POISSON and MANGENOT in *Vampyrella Closterii* during the period of digestion. It was first supposed, by analogy with the ideas accepted in animal cytology, that these vesicles, appearing when the reserve products are elaborated in the basidium and ascus and then disappearing during mitosis, might bear some relation to the formation of these reserve products. But it has been shown by observation of living material that none of these reserve products of the ascus, metachromatin, fats, glycogen, arises in these vesicles. Metachromatin is formed in the vacuoles (P. A. DANGEARD, GUILLIERMOND). Lipide granules always arise in the cytoplasm apart from the chondriosomes, as our observations have allowed us to show in fungi, such as the Saprolegniaceae and *Endomyces Magnusii* (Figs. 30, 31), where the chondriosomes are very clearly seen in living form. Glycogen also appears in the cytoplasm, as we have been able to establish by direct observation of various fungi treated with iodine-potassium iodide reagent. This reserve product is found

especially at the borders of the vacuoles and about the nuclei, where it forms as small islands which soon run together in large masses. This elaboration of glycogen takes place without any direct participation of the chondriome, a fact confirmed by DUCHAUSSOY and SARAZIN. The significance of these vesicles, therefore, is completely unknown. It will be seen that similar vesicles form where there is the slightest alteration in the cell. It might be asked, therefore, whether they are not attributable to fixatives. Yet these vesicles always appear at the same stage of development, whatever the fixative employed, and subsequently disappear. It is therefore difficult to accept this opinion, unless it be supposed that the chondriome offers a much greater fragility in that stage of development of the fungi at which they appear. Besides, we shall see that in living Saprolegniaceae similar vesicles may appear and disappear during the course of observation. There is the possibility that these vesicles indicate an elaboration of a product within the chondriosome which reagents do not reveal. The question remains unanswered for the time being.

FIG. 30. — Portions of a living filament of *Saprolegnia* showing successive stages of vesiculation of the chondriosomes. *C*, chondriosomes. *Gl*, lipide granules. *N*, nucleus.

It has been demonstrated, moreover, by our work that red pigments (carotinoids), found in the paraphyses of some Ascomycetes and in cells of some yeasts (*Sporobolomyces*), are not formed in the chondriosomes, but are always scattered in small lipide granules having no genetic relationship with the chondriosomes[1]. The pigments of the Myxomycetes are not connected with the chondriosomes either. They are phenol compounds which exist in the cytoplasm as sphaerocrystals (MANGENOT). One sees, therefore, that research on fungi, both by observation of living material and with mitochondrial methods, does not confirm the hypothesis formulated for animal cells, namely, that the chondriosomes participate in the secretory phenomena of the cells. It is seen that the rôle of the chondriosomes in plant cells still escapes us.

Physical and histochemical characteristics of chondriosomes:- It is possible, while observing living chondriosomes in the Saprolegniaceae to specify at the same time their physical and histochemical characteristics. In these fungi, as has been seen, the chondriosomes appear as mitochondria only at the terminal portions of the hyphae which will form the zoosporangia and zoospores. Everywhere else they appear as long, undulating, sometimes branched

[1]Other pigments of the paraphyses of Ascomycetes are, on the contrary, localized in the vacuoles, as is the case in *Galactinia succosa*. Let us add that some fungi also enclose in their vacuoles a yellow pigment which is a flavin, as is the case in *Eremothecium Ashbyii*.

chondrioconts. In whatever form they take, the chondriosomes appear as elements of very little refractivity, being only slightly more refractive than the cytoplasm. They are, however, always visible but are more or less clearly singled out depending upon the viscosity and density of the cytoplasm. Their visibility is sufficient for a satisfactory motion picture to have been made of them (GUILLIERMOND, OBATON and GAUTHERET).

The chondriosomes are slowly moved about by the cytoplasmic currents. Their very irregular and ordinarily extremely slow displacement may accelerate or stop brusquely and then begin again. In the course of their movements, the mitochondria generally keep their shapes but the chondrioconts modify theirs constantly. We have been able to follow the same chondriocont during a half hour (Fig. 70) and to draw all the variations in form which it undergoes. When the chondriosomes are not moving, they are usually rectilinear. During their movements, however, they may assume the most diversely sinuous forms, appearing as S, Z, propeller-shaped, etc. Their movements are reminiscent of Spirochaetes. Often the chondrioconts meet, become entangled, then separate, but we have never observed anastomosis, although they frequently branch. Their ramifications which are, moreover, transitory, are brought about by changes in shape necessitated by obstacles encountered by the chondrioconts along their path. Thus when a chondriocont suddenly encounters a lipide granule, the chondriocont branches and goes around it. The same effect can be produced by currents moving in a direction opposite to that of the chondriocont. This transitory branching, comparable to a sort of pseudopodium, shows that chondrioconts are made up of a semi-fluid and very plastic substance. They are capable of becoming shorter by thickening, or of increasing in length by growing thinner. They sometimes appear spindle-shaped or even show on their long axes small, sometimes vesiculate, swellings which afterwards disappear. These variations in form have been compared to amoeboid movements and there has been an attempt to explain them as due to modifications of surface tension (MILOVIDOV).

Under the ultramicroscope, the chondriosomes are usually invisible or just barely visible but in a few cases they may be distinguished very clearly by their slightly luminous contours, especially in very large hyphae where the cytoplasm forms only a thin layer about the vacuole. The chondriosomes are optically empty like the cytoplasm and seem to behave as a hydrogel. It may be supposed that they constitute a coacervate system in the cytoplasm, since they are not miscible with it. They can also be made to appear with a color different from that of the cytoplasm by the Zeiss micropolychromar. (Fig. 29).

The chondriosomes of the Saprolegniaceae behave as extremely fragile elements and, as FAURÉ-FREMIET observed for animal cells, the least disturbance in the osmotic equilibrium of the cell, such as a too hypotonic medium or the slightest pressure on the cover glass of the preparation, suffices to alter them. Alteration, *i.e.*,

cavulation, identical to that observed in animal cells, consists first in a swelling and increase in refractivity of the chondriosomes and then in a transformation into vesicles formed by a watery liquid within a thin, rather refractive layer which is sometimes thicker on one side. Each mitochondrium and each short rod becomes transformed into a vesicle, whereas chondrioconts of a certain length form several vesicles on their long axes[1] which are ultimately separated. These vesicles swell greatly and press against one another appearing like an aveolar structure of the cytoplasm. Sometimes they burst because of the pressure of the liquid which they enclose. Hypertonic solutions do not modify the form of the chondriosomes as long as the plasmolyzed cell is alive, but they immediately become vesiculate when death occurs.

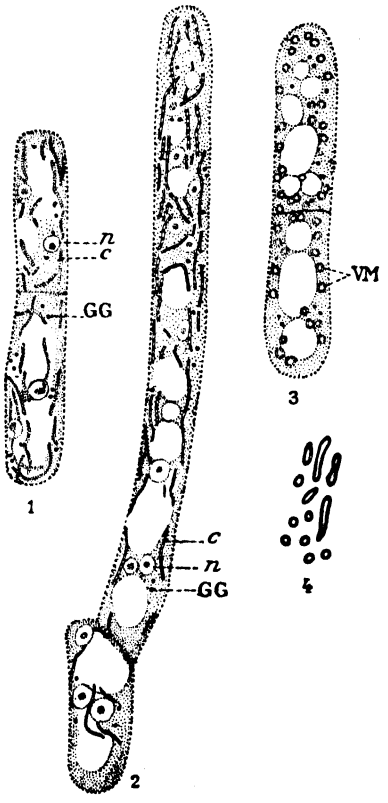


FIG. 31. — *Endomyces Magnusii* stained with Janus green. 1, oïdium; 2, germinating oïdium; 3, oïdium with vesiculated chondriosomes (*VM*); 4, stages in vesiculation of the chondriosomes. *c*, chondriosome; *GG*, lipide granules; *n*, nucleus.

For a long time chondriosomes were considered to be sensitive to high temperatures. Investigations of POLICARD, COWDRY, POLICARD and MANGENOT, carried out on animal cells as well as on plant cells and notably on the Saprolegniaceae, led to the statement that a temperature of 45-50°C. sufficed to destroy the chondriosomes almost instantaneously. More recent work by FAMIN has shown that this opinion is erroneous. In the Saprolegniaceae the chondriosomes merely become less visible at a temperature of 45-50° C., because of a modification of viscosity of the cytoplasm, and at the same time they undergo alteration: fragmentation into balls, then transformation into vesicles. A study of them after fixation shows that their chromaticity has been much diminished but that they persist until the temperature attains the point which produces coagulation of the cytoplasm, *i.e.*, about 58°C.

The chondriosomes of fungi do not stain with neutral red, cresyl blue, toluidine blue, Nile blue and methylene blue. These dyes accumulate exclusively in the vacuoles. On the contrary,

[1]This vesiculation (cavulation) seems clearly to indicate that the chondriosomes are coacervates.

they stain selectively with Janus green, methyl violet 5B and Dahlia violet. Recent research (GUILLIERMOND and GAUTHERET) has made known other vital dyes for staining chondriosomes: gentian violet, crystal violet, Hoffman violet, methyl green, iodine green, malachite green and Victoria blue. Among these, Janus green is one of the least toxic. Used with *Saprolegnia* in weak concentrations (0.0005-0.005% solutions) it stains only the chondriosomes, giving them a bluish green color in filaments which continue to show strong cytoplasmic currents. Staining is therefore clearly vital. This vital staining of the chondriosomes by Janus green is only transitory. It is observed that at the end of a few moments the chondriosomes lose their green color, whereas a rose tint appears in the vacuolar system. These observations are explained by the fact that the chondriosomes reduce Janus green to its rose derivative and this latter, having more affinity than its oxidized form for the vacuolar system, diffuses into it. If higher concentrations of Janus green are used, it stains not only the chondriosomes but also the vacuolar system. The chondriosomes then remain colored but the filaments die rapidly and do so generally without reducing the dye. At concentrations above 0.005% the dye stains the chondriosomes at first but rapidly causes them to become vesiculate, then accumulates in the vacuolar system as well, and later brings about the death of the fungus. Most of the other dyes, among them methyl violet and Dahlia violet, are more toxic and stain living chondriosomes only in concentrations not exceeding 0.005%. At greater strengths they stain the cytoplasm and nucleus as well as the chondriosomes, which become vesiculate, and then very rapidly bring about the death of the filaments. The attempts to grow cultures of Saprolegniaceae in media to which vital dyes have been added, has demonstrated the great toxicity of the dyes. It has been possible, for example, to make one culture of *Saprolegnia* grow in peptone bouillon containing up to 0.003% Janus green. Under these conditions, it first reduced Janus green to its rose derivative, which seems less toxic, and then developed without showing any coloration whatever in its chondriosomes. With a concentration of 0.004% Janus green, the fungus ceased to grow. Dahlia violet and methyl violet proved even more toxic for this same fungus which did not develop at all in a 0.001% solution of this stain.

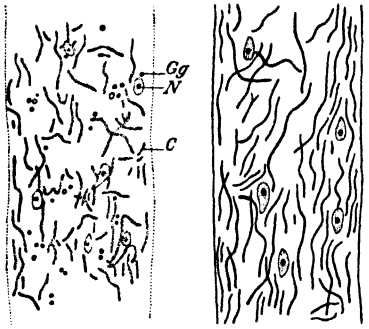


FIG. 32. — Portions of a filament of *Saprolegnia*, showing the similarity of (A) living and (B) fixed tissue. In the latter the lipide granules are not visible. *C*, chondriosomes; *Gg*, lipide granules; *N*, nucleus. Regaud's method.

The reagent iodine-potassium iodide preserves the chondriosomes perfectly. It makes them more yellow than the cytoplasm

and renders them very apparent. A 1-2% solution of osmic acid also preserves them and does not make them brown. The chondriosomes of fungi, like those of animals do not, therefore, reduce osmic acid unless followed by a treatment of pyrogallol. On the contrary, the methods of osmic impregnation recommended for bringing out the Golgi apparatus, blackens the chondriosomes very strongly and usually makes them vesiculate (GUILLIERMOND).

The comparative study of living and fixed hyphae of the Saprolegniaceae has also enabled us to demonstrate that all the ordinary fixatives containing acetic acid or alcohol profoundly alter the chondriosomes. Careful observation, however, shows that they continue to exist in the cytoplasm, sometimes in a very contracted state, sometimes vesiculate, in which case they are more stainable than the cytoplasm. The mitochondrial fixatives, *i.e.*, those of BENDA, MEVES, REGAUD, and formaldehyde as well, preserve the chondriosomes, on the contrary, as faithfully as possible in the forms they show when alive. After the action of these last fixatives, the chondriosomes stain clearly with iron haematoxylin, acid fuchsin, and crystal violet. They behave, therefore, exactly as do the chondriosomes of animal cells.

Investigations of REGAUD, then of FAURÉ-FREMIET, and of MAYER and SCHAEFFER have proved that the chondriosomes of animal cells are made up of a lipoprotein complex in which lipides (phosphoaminolipides) predominate. Of these workers, the last three named based their conclusions on the belief that mitochondrial fixatives are all oxidizing agents which transform the unsaturated fatty acids into hydroxyl acids. These are only slightly soluble in alcohol and xylol, and are capable of being strongly stained. The chromaticity of the chondriosomes, therefore, is due to the lipide substance which they contain. The work of GIROUD has shown, on the other hand, the presence of proteins in the chondriosomes. This author was able to obtain within these elements all the reactions of proteins. These facts apply as well to the chondriosomes of fungi as to those of animals. As a matter of fact, the presence of lipides in the chondriosomes is established in several ways: not only by their characteristics of fixation and staining but also by their reduction of osmic acid after treatment with pyrogallol or after prolonged infiltration in the oven; by the fact that the chondriosomes stain by the method of DIETRICH-SMITH, considered as characteristic of phosphoaminolipides; by their reaction with indophenol blue, a lipide indicator. MILOVIDOV has also demonstrated that the chondriosomes of the Saprolegniaceae give all the reactions characteristic of proteins.

It may, therefore, be concluded from their behavior, which is quite analogous to that shown by the chondriosomes of animal cells, that the chondriosomes of fungi are, like those in animal cells, composed of lipoproteins much more rich in lipides than is the cytoplasm. More recently, MILOVIDOV has established the fact, by a study of the Plasmodiophoraceae and Myxomycetes,

that these chondriosomes do not give the nuclear reaction of Feulgen and furthermore, that their protein substance has nothing in common with chromatin, contrary to the opinion expressed by P. A. DANGEARD who has called them *chromatinosomes.*

It is seen, therefore, that the study of the histochemical and histophysical characteristics of chondriosomes in the fungi have completed and made more accurate those carried out on animal cells and have shown that, in both cases, the chondriosomes behave in identical fashion.

Chapter VIII

THE CHONDRIOME (Continued)

The chondriome and its development in the phanerogams. Relationships between chondriosomes and plastids. The facts:- The first investigations on this subject were those of PENSA (1910). Applying the Golgi method to various tissues of phanerogams, *i.e.*, impregnating sections of living phanerogam tissue

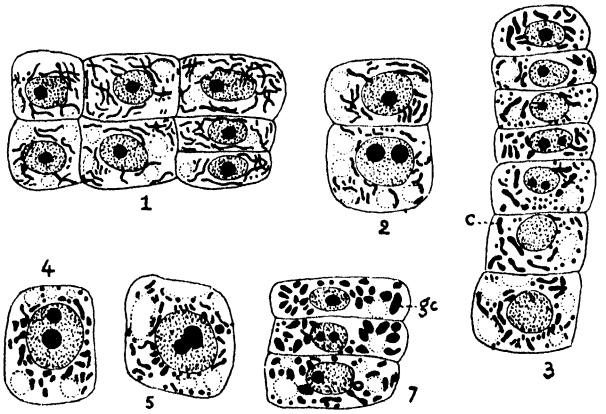


FIG. 33. — Development of chloroplasts in a young leaf of the plumule of barley. 1, chondriome in the basal meristem; 2, cells beginning to differentiate with some elements of the chondriome showing thickening, and 3-5 their transformation into chloroplasts; 7, cells in older regions showing chloroplasts. *c*, young chloroplasts; *gc*, mature chloroplast. Regaud's method.

with silver nitrate followed by treatment with a reducing solution of hydroquinone, he noticed that the chloroplast has the property of reducing silver nitrate and appears strongly blackened by a deposit of metallic silver on its substratum. Now, while studying the chloroplasts in differentiating tissues, PENSA stated that these elements appear first as very small bodies which present the form characteristic of chondriosomes in animal cells. Yet this author never reached the point of specifying the origin of these chondriosome-shaped elements. He did not find them in tissues lacking in chlorophyll and stated that their property of reducing silver nitrate is correlated with the presence of chlorophyll in the substratum. PENSA, however, put forth the hypothesis that the chloroplasts are derived from chondriosomes on which chlorophyll accumulates, giving them the property of reducing silver nitrate.

Without knowledge of PENSA's work, LEWITSKY, a student of STRASBURGER, was working at the same time with mitochondrial technique (method of MEVES). LEWITSKY (1911) showed in the bud of *Asparagus officinalis* that the chloroplasts are built up from minute elements looking like the chondriosomes of animal cells. This investigator concluded therefore that the plastids, contrary to the opinion of SCHIMPER, do not keep their individuality but arise from chondriosomes which LEWITSKY considers originate, in turn, from a differentiation of the cytoplasm.

At the same period (1911) and a little later, in cells of plants belonging to very diverse groups (phanerogam seedlings, nucellus, embryo sac, pollen, asci of *Pustularia vesiculosa*), we were demonstrating by Regaud's method, the existence of chon-

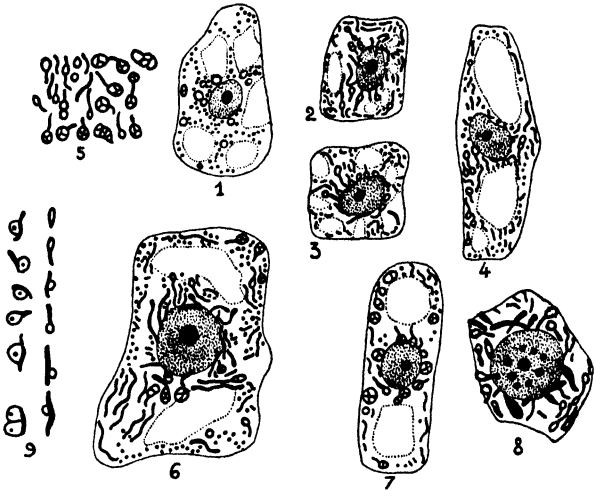


FIG. 34. — Various types of starch formation. 1, within mitochondria in young potato tuber; 2-4, compound grains within chondrioconts in the meristem of a young root of castor bean; 5-7, compound grains within chondrioconts in bean root; 8, within fusiform leucoplasts surrounding the nucleus; 9, leucoplasts showing successive stages in starch formation. 8, 9, from the root of *Phajus grandifolius*. Regaud's method.

driosomes quite similar in form, as well as in histochemical behavior, to those of animal cells. Our investigations led us to consider, contrary to the opinion of LEWITSKY, that the chondriosomes are permanent organelles, being transmitted by division from cell to cell and incapable of forming *de novo*. We were demonstrating besides, by a study of the plumule of barley, that chloroplasts arise by the differentiation of some of the chondriosomes in cells of the meristem. Finally by a study of the potato tuber and of roots of various seedlings, notably those of castor bean, we were able to prove that starch never forms in the cytoplasm but is always the product of the activity of chondriosomes.

Our later investigations (1912-1923) as well as those of PENSA (1912) and LEWITSKY (1912), followed by many others, confirmed these facts and if interpretations still differ, it nevertheless seems

that the great majority of cytologists are at present in agreement in recognizing that the plastids of SCHIMPER are derived from elements presenting the same forms as those of the chondriosomes.

The life history of the chondriosome in phanerogams will now be studied in detail by first following the formation of chloroplasts. As the phenomena are the same in all buds, it is sufficient to choose a single example. The most favorable, because of the disposition of foliar primordia, is the bud of *Elodea canadensis*, first investigated by LEWITSKY. Afterward, it was the object of intensive study for us and our reports were confirmed by FRIEDRICHS.

If a longitudinal section of a bud of *Elodea canadensis* be examined after being fixed by Regaud's method (fixation by a mixture of potassium bichromate and formaldehyde, and staining with iron haematoxylin), there may be observed in the meristem of the stem and in the youngest foliar primordia, a chondriome exactly like that of many animal cells, composed of a mixture of chondriosomes and granular mitochondria. These elements have a diameter of about 0.5-1μ. (Fig. 36).

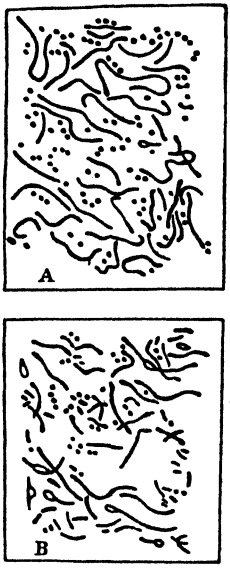


FIG. 35. — A comparison (A) of the chondriome of the vegetative point of *Elodea canadensis* with (B) that of the liver of a mouse. Regaud's method. × 3,000.

By following successively developed foliar primordia, there may be seen with the greatest accuracy, all the developmental stages of the chondriome and it may be observed that the chondrioconts differentiate into chloroplasts. The differentiation, manifested by a thickening of the chondrioconts, begins in those foliar primordia which are about 160μ long. In those measuring about 200μ in length, the chondrioconts form little swellings on their long axes in which a small starch grain is sometimes elaborated. As this grain is not stained by iron haematoxylin, it looks like a vesicle. Starch grains thus formed are only transitory and soon disappear. The swellings then gradually separate by rupture of the slender portions between them. They increase in volume and, in mature cells, take on the appearance of large, rounded or ovoid, chloroplasts about 4-8μ in diameter. These are distinguished from the chondrioconts, from which they arose, by the modification which they have undergone in their chemical qualities which gives them a special resistance. They are preserved by all the fixatives which destroy the chondriosomes. Henceforth these chloroplasts often elaborate large starch grains.

During the differentiation just described, the granular mitochondria elongate first into rods then, in mature cells, generally become typical chondrioconts.

In the axil of each leaf primordium there is found, as is known, a small scale made up of a group of cells in which there is no pro-

duction of chlorophyll. In these cells, the chondriome always keeps the characteristics which it shows in the meristem. It, therefore, remains undifferentiated, made up of a mixture of mitochondria and thin chondrioconts.

These phenomena may be verified in fresh material by studying

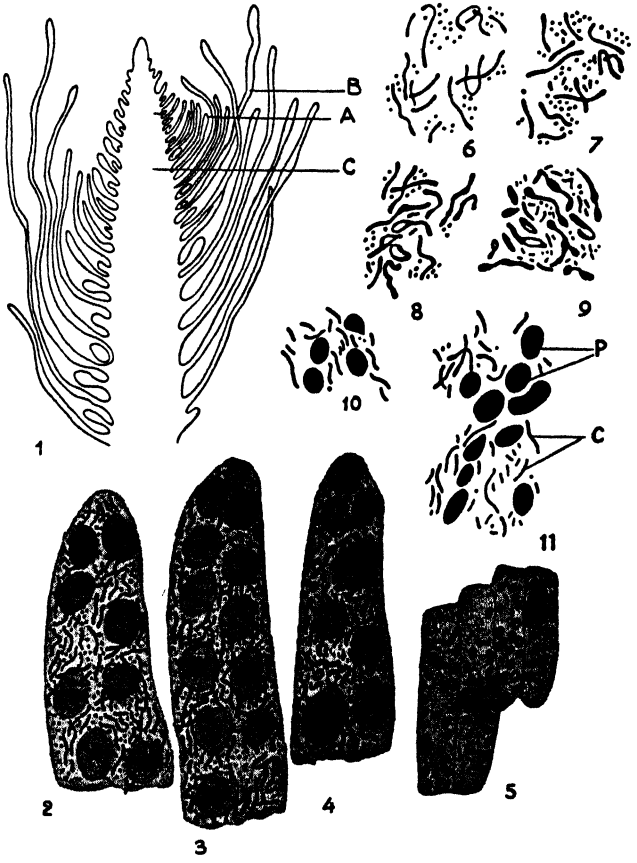


FIG. 86. — Development of the two categories of chondriosomes in the bud of *Elodea canadensis*. 1, diagram of longitudinal section of bud; 2, mitochondria and chondrioconts in a foliar primordium at level (*A*); 3, 4, chondrioconts transforming into chloroplasts in a slightly older foliar primordium; 5, mature chloroplasts in cells of a nearly mature leaf taken at (*B*). Some of the mitochondria have become rods or chondrioconts; 6-11, details showing the same sequence of events. *C*, level at which chloroplasts develop in the stem, also chondriosomes in Fig. 11. Regaud's method.

the living bud. The tip of the bud may be seen, without any alteration taking place within its cells, by stripping it of its oldest leaves and putting it in water under a cover slip which is pressed gently so as not to injure the vegetative point. It is seen that the meristem of the stem and of the youngest foliar primordia do not contain chlorophyll. In addition to a large nucleus, these cells show only a confusedly granular cytoplasm in which it is possible

to distinguish the chondriome. It is only in foliar primordia in which the chlorophyll is beginning to appear that the chondrioconts are visible. They are here impregnated with chlorophyll and all the forms can be followed in sequence from these elements to the large chloroplasts of mature cells. The other chondriosomes, however, are difficult to distinguish. The cells of the mature leaves are, on the other hand, very transparent and very favorable for

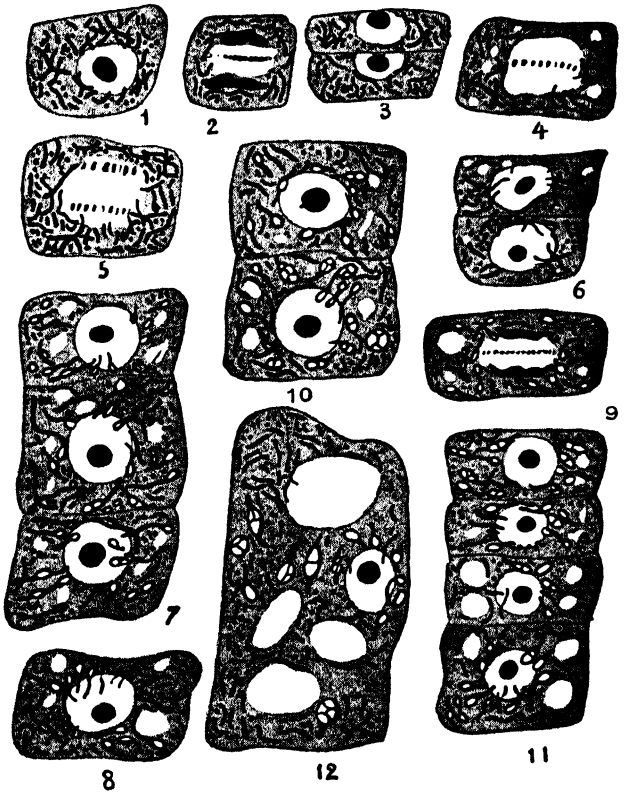


FIG. 87. — Development of the chondriome in the castor bean root. 1-6, meristem; 7-11, differentiating cells, plastids forming starch; 12, differentiated parenchyma cell of the central cylinder. Regaud's method.

the study of living cells. In them can be seen all that Regaud's method brings out and it is possible to distinguish with great clearness within the hyalin, homogeneous cytoplasm, large chloroplasts, often in the process of dividing, interspersed with chondrioconts whose slightly higher refractivity distinguishes them from the cytoplasm.

It is easily possible to follow the formation of chloroplasts by a study of the development of the chondriome in other buds, among them barley which was the object of our first research (1911). Here also, the chloroplasts are derived from chondrioconts which thicken, and on their long axes form small swellings which after-

wards separate and then elaborate a grain of transitory starch. It is not until this is absorbed that the swellings increase in volume and take on their characteristic appearance of large chloroplasts.

The living root of *Elodea* does not lend itself to study. On the other hand, Regaud's method brings out in the meristem a chondriome entirely similar to that of the vegetative tip. During the differentiation of tissues all that can be observed is that a certain number of elements of the chondriome, especially the chondrioconts, without modifying their form or chemical quality, elaborate little starch grains along their long axes, but this elaboration is not very active. When the root is exposed to light, on the contrary, there are formed in the course of cellular maturation and by differentiation of a part of the chondriosomes, chloroplasts similar to those in the stem and leaves.

A study of the root of the castor bean (Figs. 37, 38) is more profitable and will serve as an example here. The chondriome of cells of the meristem is, here also, composed of a mixture of granules, rods and chondrioconts. In the central cylinder, a part of these elements, especially the chondrioconts, elaborate small starch grains directly. On the long axis of the chondrioconts, there are seen to form small vesiculate swellings occupied by a sort of vacuole which corresponds actually to a starch grain left colorless by Regaud's technique. Soon, around this small starch grain, others are seen to appear which give a spongy appearance to the swellings and thus a compound starch grain is produced. This increases in size little by little while remaining surrounded by a thin mitochondrial layer prolonged to a sort of tail, the remaining portion of the chondriocont. In the cells of the cortex, on the contrary, some of the elements of the chondriome differentiate by thickening slightly and it is not until after this is accomplished that they elaborate starch as first described. Thus, some of the elements of the chondriome elaborate starch and play the rôle of amyloplasts either immediately, or after thickening slightly. It is easy to obtain the characteristic reaction for starch by treating the preparation obtained by Regaud's method with the reagent iodine-potassium iodide. The chondriocont is stained by the haematoxylin while the starch grain becomes yellowish brown, due to the action of the xylol which turns yellow the

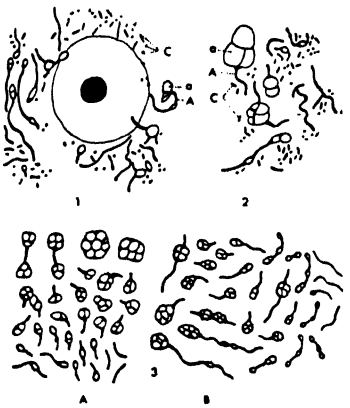


FIG. 38. — 1,2,3A, the chondriome in the castor bean root. 3B, in the bean root. 1, portion of a parenchymatous cell of the central cylinder showing the nucleus surrounded by chondriosomes (*C*) and amyloplasts (*A*); 2, amyloplasts containing compound starch (*a*) in a similar cell; 3, less highly magnified stages in the formation of starch by the chondriocont-shaped amyloplasts.

starch grains stained by the iodine. Moreover, simultaneous staining of the starch and the chondriocont may be obtained by various more complicated processes. MILOVIDOV, especially, has shown how to make such permanent preparations. These methods are much more delicate and do not give constant results.

Starch formation takes place in the same way in the greatest variety of tissues which are without chlorophyll: roots, tubers, epidermis. Yet there are cases, as in the tuber of the potato, in which the chondriome is represented only by mitochondria which elaborate starch after having undergone a slight increase in volume. In such cases they take on the appearance of vesicles, due to the production in their interior of a starch grain which mitochondrial methods do not stain. Sometimes the chondrioconts which will later elaborate starch may acquire, before its production, a much more marked increase in volume which makes it possible to distinguish them very clearly from the other chondriosomes in the mature tissue. This is seen, for example, in the root of *Phajus grandifolius* in which by following the meristem to the region of differentiation, it is seen that some of the chondrioconts take the form of rods or spindles. These chondrioconts are very clearly bigger than the chondriosomes which continue to exist side by side with them but without increasing in size. These enlarged elements correspond to the amyloplasts described by SCHIMPER and MEYER, through the agency of which the grains of starch arise. It seems that the increase in volume is due to the formation in the chondriocont of a needle-shaped protein crystal lying along the long axis of the element, whose contours follow that of the crystal. In other cases the amyloplasts assume the appearance in mature cells of rather long rounded bodies (hairs of *Tradescantia virginiana*). It may be added that the simple or compound starch grains instead of arising in the center of the swelling of the chondriosome, chondriocont, or mitochondrium, may form on its periphery. The chondriosome then bears a vesicle whose wall is much thicker on one side than on the other. The starch grain which occupies the vesicle increases in size and ends by bursting out of the chondriosome which is thus reduced, little by little, to a thin cap, covering the starch grain in the region most distant from its hilum (potato tuber, root of *Phajus grandifolius*). The starch grain thus formed no longer remains surrounded by a continuous mitochondrial layer as in the preceding case (Figs. 40, 41).

FIG. 89. — Stages in starch formation in potato tubers.

It is difficult to check these phenomena by observation of living material. Roots in general do not lend themselves to this type of investigation. On the other hand, in the course of our research, we have found exceptionally favorable examples in which the entire process of elaboration of starch can be followed with remarkable accuracy in living cells. In a fragment of the epidermis of the anther of a young flower of *Iris germanica* examined in Ringer's solution, a chondriome is observed with great clearness, composed of thin, elongated, and undulating chondrioconts which sometimes branch, interspersed with granular mitochondria and short rods. In some cells there is no elaboration of starch; in others there may be seen several stages in the formation of small, compound, very refractive starch grains on the long axis of the chondrioconts. Similar phenomena may be observed in epidermal cells of the leaves, of the bracts, and of all very young floral parts. At later

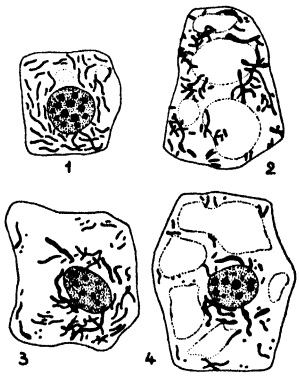


FIG. 40 (left). — Successive stages in the formation of leucoplasts in the root of *Phajus grandifolius*. Regaud's method.

FIG. 41 (right). — Leucoplasts from the root of *Phajus grandifolius* showing starch. 1, central cylinder; 2, cortical parenchyma. Regaud's method.

stages of development it is seen that the starch grains are absorbed within the chondrioconts which persist after the disappearance of the starch (Fig. 44). It can also be seen in these same cells that the chondrioconts are, at certain stages, the seat of a production of small osmium-reducing lipide globules, clearly visible on the long axis of these elements because of their strongly refractive power. These granules which often completely fill the chondriocont are very frequent in the monocotyledons. They can not be considered as formed of α-β hexylene-aldehyde (MEYER), for they present characteristics of lipides and not those of aldehydes. The fact that they are stained by Dietrich's method suggests that they are made up of phosphoaminolipides. These granules, very numerous in the young stages of development of leaves, bracts and floral parts, disappear from the plastids as soon as the starch grains and pig-

ments begin to form, except, however, in certain regions where they persist during the entire life of the cells. Do they represent an intermediate product from which starch and pigments are built up, or do they result from a breaking down of the lipoprotein complex which makes up the plastids (lipophanerosis)? It is difficult to say. In any case, these granules reappear in large numbers in the plastids at the moment when the flower begins to form. They are in this case products of disintegration of the plastids and mark the beginning of plastidial degeneration.

The epidermal cells of perianth parts of the tulip are also particularly favorable objects for observation of the living chondriome and in them it is possible to follow the formation within the chondrioconts of a yellow pigment, xanthophyll. In the white tulip, for example, the chondriome can be observed very clearly in a frag-

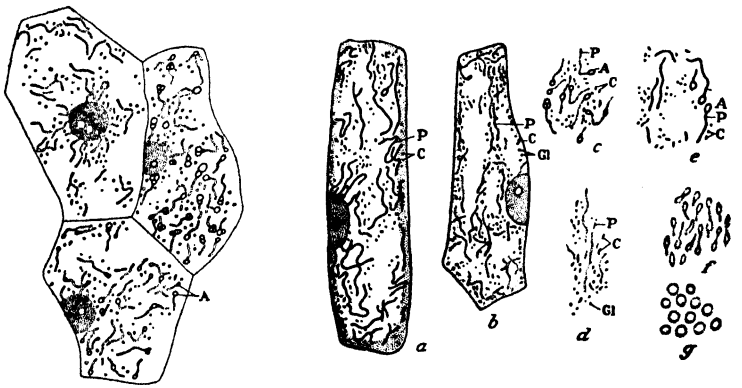


FIG. 42 (left). — Epidermal cells of living young anther of *Iris germanica*, showing refracting mitochondria, chondrioconts and strongly refracting lipide granules. The two lower cells contain chondrioconts with small compound starch grains (*A*) on their long axes.

FIG. 43 (right). — Epidermal cells of leaves of *Iris germanica*. A comparison of the chondriome in (*b*) a living cell with (*a*) one fixed by Regaud's method, showing that the chondrioconts in (*a*) correspond to the plastids in (*b*); that the rod-shaped and granular chondriosomes are similar in the two cells; that the lipide granules appear only in (*b*). *c, d, e*, similar portions of the cell showing (*c, e,*) starch bearing plastids in living and fixed material (Regaud's method) respectively; *d*, plastids not forming starch; *f, g*, successive stages in the vesiculation of living plastids. *C*, chondriosomes; *P*, chondriocont, plastid; *Gl*, lipide granules; *A*, starch.

ment of the epidermis of the perianth. It is made up of a considerable number of very elongated chondrioconts and granular mitochondria. The bases of the perianth parts are almost always yellow and, on examining the epidermis in this region, it is seen that it is the chondrioconts which serve as substratum for the xanthophyll pigment and consequently represent the chromoplasts. The mitochondria, on the contrary, remain colorless. In yellow flowers, however, the chondrioconts in all parts of the epidermis appear yellow because of the xanthophyll.

In living epidermal cells of the perianth and those of the exocarp of fruit of monocotyledons, the chondriosomes can be observed with greatest ease and the formation of carotinoid pigments fol-

lowed. A study of the latter can hardly be made from preparations where the mitochondrial technique has been used, for, although the plastids are stained, there is no indication as to what pigments they contain. So in the flower of *Clivia nobilis*, it can be seen that the orange-red pigment, carotin, arises directly from chondrioconts. Small starch grains are first elaborated and, at the moment when these are absorbed, the carotinoid pigment arises in the interior of the chondrioconts as small grains or more especially as long needle-shaped crystals. In the flower of *Sternbergia* the chondrioconts form several acicular crystals of carotin which give them the appearance of thick spindles. In other cases, the chondriosomes in which the pigment will form are always chondrioconts

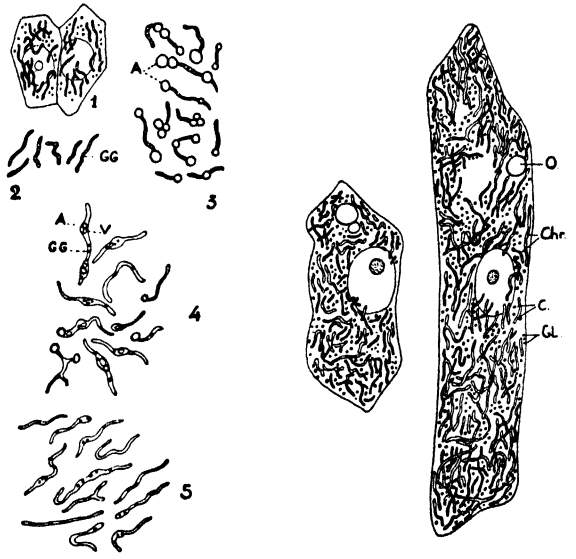
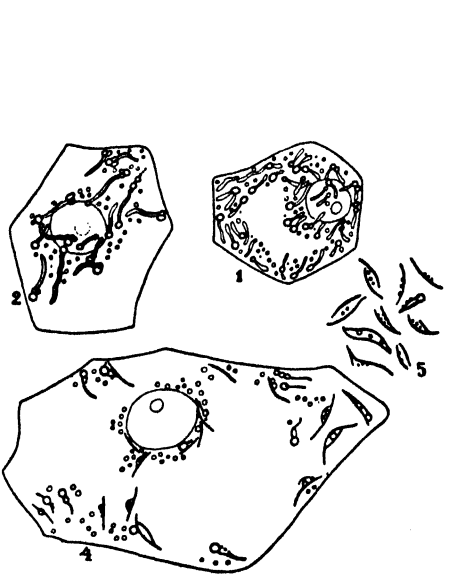


FIG. 44 (left). — Development of leucoplasts in living epidermal cells in leaves of *Iris pallida*. 1, lipide granules (*GG*) within the leucoplasts (chondrioconts) in a young leaf; 2, detail of leucoplasts; 3, later stage, leucoplasts containing starch (*A*); 4, absorption of starch, diminution in lipide granules; 5, leucoplasts without starch containing lipide granules in adult leaf.

FIG. 45 (right). — Living epidermal cells of a petal of white tulip. At left, from a young flower; at right, from a mature flower. *C*, chondriosomes; *Chr*, chromoplasts; *GL*, lipide granules; *O*, fatty body.

enclosing small starch grains. When these are absorbed, small vesiculate swellings enclosing a watery liquid are formed on the long axis of the chondriocont. Small grains of carotin appear on the walls of these vesicles which later become isolated by rupture of the slender regions of the chondriocont which separate them. They then appear as small rounded vesiculate chromoplasts (pericarp of the fruit of *Asparagus officinalis* and of *Arum italicum*). In the epidermis of the perianth of *Iris germanica* the phenomena are a little more complicated. The yellow pigment, xanthophyll, first appears in a diffuse state in the chondrioconts which contain small

starch grains. Then, when the starch is absorbed, the chondriocont thickens at the same time that large vesicles appear along the element. These may disjoin by a rupture of the more slender portions of the chondriocont which connects them, so that vesicular chromoplasts are formed with tails of varying lengths. In other cases the pigment begins to appear in chondrioconts which increase their dimensions proportionally as the pigment develops, until they have been transformed into large chromoplasts of the same form and dimensions as chloroplasts.



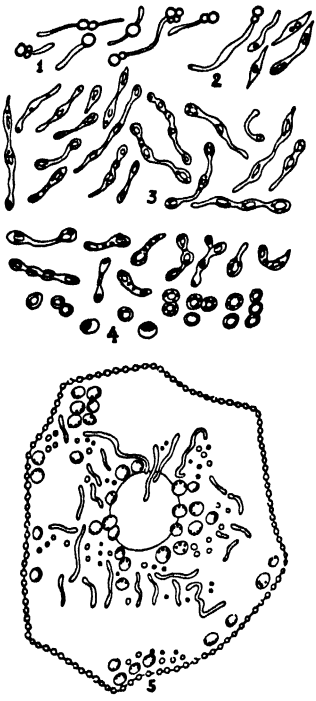


FIG. 46 (left). — Transformation of chondrioconts into chromoplasts in living epidermal cells. 1, formation of starch in very young petals; 2, starch being absorbed and replaced by small granules and needle-shaped carotinoid pigment in older petals; 4, the same in an open but young flower; 5, chromoplasts in an older flower. 1, 2, *Clivia nobilis*. 4, 5, *C. cyrtanthiflora*.

FIG. 47 (right). — Development of chromoplasts in living cells of the fruit of *Asparagus officinalis*. 1, chondrioconts forming starch; 2, starch being absorbed; 3, carotin granules forming on the borders of vesiculate swelling in the plastids; 4, fragmentation of chondrioconts to form round vesiculate plastids containing carotin granules which tend to fuse; 5, chromoplasts and chondriosomes in a cell of the pericarp of a nearly ripe fruit.

Xanthophyll always seems to be diffuse in the substratum of the plastid or else in the state of indistinct granules. Its isomer, rhodoxanthin, on the contrary, appears as isolated, clearly distinguishable granules. This is true of carotin and lycopin if they are not in crystalline form. When crystalline, the crystals give widely-differing shapes to the chromoplasts. These facts show that whenever the chromoplasts do not arise by metamorphosis of the chloroplasts as in the parenchymatous tissue, studied especially by SCHIMPER, MEYER, and COURCHET, they arise from chondrioconts which have first elaborated starch.

The very best material for the study of living cytoplasm is to be found in the epidermal cells of flowers and various organs of the monocotyledons, those of *Iris* and tulip among others, as well as in the bulb scales of *Allium Cepa,* which will be taken up later, and in the Saprolegniaceae which have just been studied. On these forms we have been able to make the most accurate observations of the chondriosomes that it has been possible to make up to the present time.

We have been able to show, by a comparison of these observations with those on fixed and stained cells, that the mitochondrial methods preserve the cytoplasm and its morphological constituents, the chondriosomes and plastids, in a manner as faithful to the form they present in life as it is possible to have it done. These observations permitted us, also, to specify the histochemical and histophysical characters of the elements. This will be taken up later.

We have studied the chondriome very accurately during the formation of the embryo sac and of pollen grains in the Liliaceae and, in particular, in *Lilium candidum.* In the young ovary, all the cells of the nucellus present a chondriome made up of a mixture of chondrioconts and of mitochondria. The embryo sac, which arises from a cell of the nucellus, first shows a chondriome similar to that of other cells of the tissue, then, in the course of its differentiation, at the moment when synizesis begins, it is observed that a part of the chondrioconts thicken and form small swellings on their long axes. These grow little by little, often detach themselves from the chondriocont in which they rise by rupture of the thin portions which connect them, then enlarge greatly, and take on a crystalline appearance. This seems to be due to the production in their interior of protein crystalloids. These plastids, which we have called *proteoplasts,* because of their ability to elaborate protein, then appear to be digested in the cytoplasm and their protein is thus utilized as a reserve product. In the synergids, the egg and the antipodal cells, on the contrary, no elaboration of protein is noted and the chondriome remains about as it is in the embryo sac at the beginning of differentiation, except for the appearance of small granules slightly larger than the other elements of the chondriome. These granules are the leucoplasts. In the tulip no proteoplasts are observed and

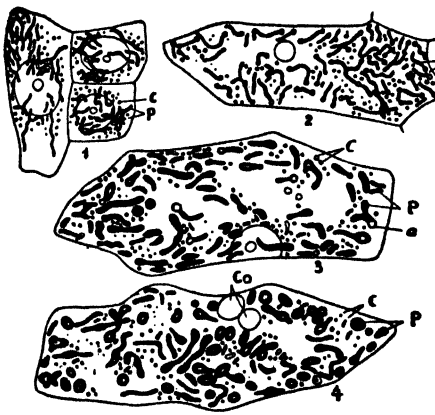
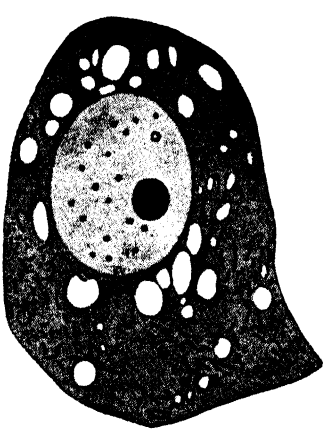


FIG. 48. — Development of chromoplasts in cells of the petals of *Iris germanica.* 1, very young stage showing colorless chondriocont-shaped plastids and granular chondriosomes; 2, the starch-bearing plastids begin to fill with xanthophyll; 3, the starch is absorbed as the chromoplasts increase in size; 4, mature cell with variously shaped chromoplasts, most of them showing vesiculate swellings.

the chondriome, consisting of a mixture of chondrioconts and mitochondria, undergoes no modifications during the development of the embryo sac[1]. (Figs. 49, 50, 51, 52).

In the sporogenous cells of the pollen grains of *Lilium candidum*, the chondriome is seen clearly as short rods and granules. In the pollen mother cells, only mitochondria are to be found. Beginning with the period of synizesis, some of these mitochondria which are to become amyloplasts, undergo a slight increase in size, then, at the time of the heterotypic mitosis, they elongate into chondrioconts and afterwards, in the pollen grains, break up into mitochondria. The remainder of the mitochondria are unchanged from the beginning. When the pollen grain is mature, only gran-



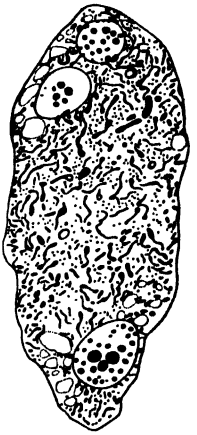


FIG. 49 (left). — Embryo sac of *Lilium candidum* at the beginning of differentiation. × 1500. Regaud's method.

FIG. 50 (right). — Formation of proteoplasts in the embryo sac of *Lilium candidum* at the end of the second mitosis. Regaud's method.

ular mitochondria are found, among which a few larger than the others elaborate compound starch grains.

Other investigators of the development of the chondriome during the formation of pollen in other plants have produced data more or less analogous (WAGNER, MASCRÉ, KRJATCHENKO-DOUZE, PROSINA, Mrs. LUXEMBURG, KRUPKO, Miss PY). Recently there has appeared LEWIS ANDERSON'S very good work on the development of pollen in *Hyacinthus orientalis*. Investigations of NICOLOSI-RONCATI, WAGNER, and others have shown that the chondriosomes in the spore mother cells of some species may collect in a compact mass which surrounds the spindle as a sort of mantle during the heterotypic division and divides (*chondriocinesis*) at the same time as the nucleus. The significance of this grouping of chondriosomes is not clear and one wonders if it does not correspond to an alteration.

Chondriosomes have been observed in all the cells of the embryo before the maturation of the seed and in the seed in the dor-

[1]LEWIS ANDERSON finds this is also the case for the embryo sac of the hyacinth.

mant state (GUILLIERMOND, WAGNER). Some of the chondriosomes later, at germination, form the amyloplasts of the root and the chloroplasts of the chlorophyll-bearing organs (leaves, etc.). It has been proved by our research that chondriosomes exist permanently in all phanerogam cells and that they are transmitted by division from one cell to the next.

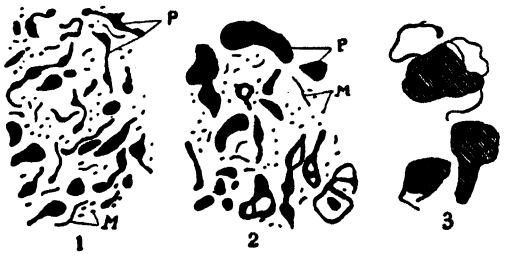
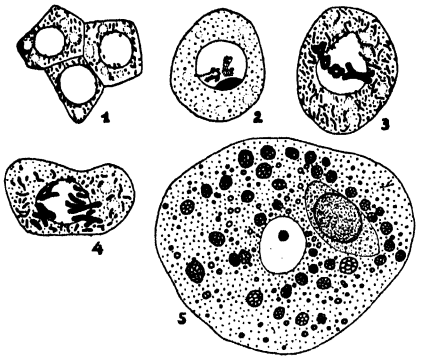


FIG. 51. — Portion of the embryo sac of *Lilium candidum*. 1, 2, stages in the development of the plastids (*P*) and the mitochondria (*M*); 3, digestion in the plastids.

The origin of plastids, which for so long remained obscure in the phanerogams, is now well known, through the use of mitochondrial techniques, by means of which a chondriome has been demonstrated in embryonic cells analogous to that in animal cells. The entire life history of this chondriome has been followed and it has been shown that events take place as if the plastids arose by differentiation of some of the elements of the chondriome.



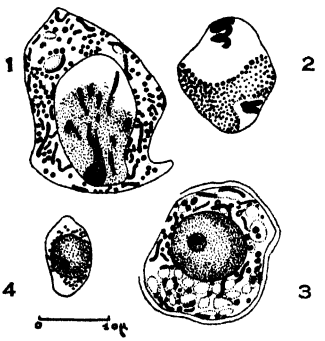


FIG. 52 (left). — Development of the chondriome during the formation of pollen grains in *Lilium canadense*. 1, sporogenous cells; 2, spore mother cell in synizesis, leucoplasts appear slightly larger than other chondriosomes; 3, metaphase, leucoplasts have become chondrioconts; 4, anaphase; 5, pollen grain, leucoplasts slightly larger than other chondriosomes, various stages in development of compound starch grains.

FIG. 53 (right). — Chondriome in pollen of *Helleborus foetidus*. 1, synizesis; 2, accumulation of chondriosomes about the nuclear figures of the first division; 3, pollen grain; 4, generative cell. (After Miss PY).

There is still one gap in our knowledge. This is the behavior of chondriosomes during fertilization. It is still not known whether the chondriosomes of male origin participate in this phenomenon. In a recent work, however, LEWIS ANDERSON reports having ob-

served that in *Hyacinthus orientalis* the facts are in favor of a passage of the chondriosomes of male origin into the egg. KIYOHARA, almost at the same time, described in certain phanerogams the passage of plastids from the pollen tube into the oosphere. More recently still, MANGENOT in gymnosperms (Pine) was able to follow the course of the chondriosomes of male origin because of their size which is greater than that of the chondriosomes of the oosphere. All the chondriosomes of the pollen tube and oosphere are in the form of mitochondria but those in the pollen tube are larger, and can be followed during their penetration into the oosphere during fertilization, and can be recognized after fertilization has taken place. During the development of the embryo, however, the chondriosomes of male origin remain in that portion of the oosphere which does not contribute to the formation of the embryo and which will later degenerate. Therefore there does not seem to be any mixing of chondriosomes of male and of female origin.

Chapter IX

THE RELATIONSHIP BETWEEN CHONDRIOSOMES AND PLASTIDS

Interpretations:- It was logical to admit from the facts already displayed, which have been verified by many observers, that the plastids described by SCHIMPER arise by differentiation of some of the elements of the chondriome during cellular development. This opinion, which had been maintained from the beginning by many workers, notably PENSA (1910), LEWITSKY (1912-1913), FORENBACHER (1912), MAXIMOV (1913), and which we ourselves were among the first to formulate in our early work, is still held by LEWITSKY and his school (1925-1927), ALVARADO (1918-1925), MOTTE (1928), GATENBY and his collaborators (1930), JUTTA VON LOUI (1930), CHALAUD (1923), LEWIS ANDERSON and others. It was adopted by WILSON in his book, *The Cell in Development and Heredity* (1925). Still the opinion has been variously expressed. Thus, for certain investigators such as LEWITSKY and RANDOLPH, the chondriosomes are not permanent components of the cytoplasm but form *de novo* from it. Our first interpretation was quite contrary to this. Not having recorded any fact which would permit us to think that the chondriosomes can arise by differentiation from the cytoplasm, we had from the beginning considered them as permanent components of the cell, incapable of forming otherwise than by division of pre-existing chondriosomes. Therefore at that time, we considered the plastids of the phanerogams as a variety of chondriosome, differentiated in the course of cellular development and having a special function. The plastids, therefore, we believed belonged to a much more general category present in every cell, whether plant or animal. Then too, the theory that we had formulated was only an extension of that of SCHIMPER and MEYER and in no way contradicted it. It is this same point of view which MEVES and ALVARADO adopted.

Although based on incontestable facts, this theory, however, raises very serious theoretical difficulties, for it can only be applied to higher plants. In fact, although in the phanerogams the origin of plastids had been for a long time only imperfectly known, this was not so for the algae. In many of these plants, as has already been said, the chlorophyll is present in all stages of plant development and in that case chloroplasts are observed in all cells. These chloroplasts are transmitted by division from cell to cell, beginning with the egg. This has been well known since the work of SCHMITZ. We have seen, besides, that in many algae there is in each cell only a single, voluminous chloroplast which divides at each cellular division. This chloroplast, however, can not be considered as different from the chloroplasts of the phanerogams, for it offers the same histochemical characteristics. There are found, more-

over, in the algae, bryophytes, and pteridophytes, all the intermediate stages between this chloroplast of special form and chloroplasts such as exist in the phanerogams. Now the research of RANDOLPH has demonstrated in *Vaucheria*, which contains chloroplasts similar to those in phanerogams, that these chloroplasts are found in all parts of the thallus at the same time as the chondriosomes. Our work on *Spirogyra* showed the chondriosomes to be constantly present and distinct from the single permanent chloroplast which is characteristic of this alga. The work of SAPĚHIN, of SCHERRER, and of MOTTIER showed that in the bryophytes, too, chlorophyll seems to persist in all stages of development, and that all cells, even the egg and apical cell of the vegetative shoot, contain both chloroplasts and chondriosomes. There even exists, in this group, the genus *Anthoceros* in which each cell contains only one single crescent-shaped chloroplast adhering to the nucleus. Coexistent with this organelle there are, however, numerous chondriosomes. There can not, therefore, be found in these plants any genetic relationship between the chloroplasts and the chondriosomes. It is to be added that in centrifuging the cells of various algae and cells of *Elodea canadensis* in the process of division, BOROVIKOV was able to obtain cells without chloroplasts but containing chondriosomes. He was never able to observe in these cells any transformation of chondriosomes into chloroplasts. These contradictory facts therefore must be explained.

FIG. 54. — Portion of a filament of *Vaucheria;* division figures of the chloroplasts (*C*) and of the chondriosomes (*M*). (After MANGENOT).

RUDOLPH (1911), SCHERRER (1913), SAPĚHIN (1913), ARTHUR MEYER (1914-1921) and NOACK (1921) protested, not altogether disinterestedly, against the new results obtained in the phanerogams by mitochondrial technique which, at least in appearance, seem to invalidate the classical theory. They did not hesitate to consider the chondriosomes and plastids as inherently different formations. To explain the phenomena in the phanerogams, RUDOLPH, SCHERRER and SAPĚHIN report that in the meristematic cells of these plants, the plastids and the chondriosomes stain in the same way with mitochondrial technique. According to these investigators, the plastids appear as small grains and the chondriosomes as rods or filaments. Now, as the plastids divide actively, RUDOLPH, SCHERRER, and SAPĚHIN believe they become dumb-bell shaped and are thereby confused with the chondriosomes (chondrioconts). But from the moment that the cells differentiate, the plastids enlarge and appear as large bodies which it is no longer possible to confuse with the chondriosomes, since the latter keep their original form

and dimensions. SCHERRER and SAPĚHIN think, furthermore, that chondriosomes are not permanent elements of the cytoplasm but that it is more probable that they are merely reserve products. MEYER, who was the instigator of this opinion, attributed to the chondriosomes a ferronuclein-like constitution and called them *Allinantes*. NOACK has carried this idea further and maintains that there is not the least morphological or histochemical resemblance at any time between chondriosomes and plastids. He finds chloroplasts even in the meristematic cells of buds of *Elodea canadensis* and shows them to be different histochemically from the chondriosomes, for they are preserved by all the fixatives which destroy chondriosomes.

JARETZKY, in his German edition of SHARP's book, *Einführung in die Zytologie* appears to be of the same opinion, an opinion expressed, moreover, with insufficient knowledge of the question. GEITLER takes the same stand (*Grundriss der Zytologie*) and also

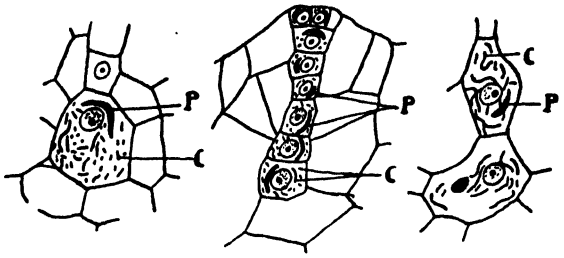


FIG. 55. — *Anthoceros*. *C*, chondriosomes; *P*, plastid. (After SCHERRER).

KÜSTER. The latter, particularly in *Die Pflanzenzelle*, tends to consider the chondriosomes as heterogeneous formations resulting from cellular metabolism.

P. A. DANGEARD, having made observations exclusively on living material, first thought, as will be shown further on, that the formations described as chondriosomes belonged to the initial forms of the vacuolar system (vacuome, p. 149) and that the refractive granules corresponded to the microsomes of early authors. These are encountered in all cells and will be discussed later (p. 203). According to DANGEARD, the plastids, therefore, bear no relation to these dissimilar formations, the chondriosomes. However, after more profound studies with mitochondrial technique, DANGEARD was obliged little by little to renounce his former opinion. He had to recognize that the chondriosomes, which he at first had found impossible to distinguish from the leucoplasts, are discrete elements corresponding neither to young vacuoles nor to microsomes, and that they evidently very much resemble the leucoplasts[1].

[1] P. A. DANGEARD, who, however, is not convinced as to the individuality of the chondriosomes, says in his last re-statement of the question with reference to the multiplication of the chondriosomes, "If this multiplication were to take place by division or fragmentation, the chondriosomes would be akin to the plastids but distinguishable from them".

MOTTIER (1918) finds that plant cells containing chlorophyll constantly enclose plastids and chondriosomes which stain in the same way. In meristematic cells of phanerogams he finds these two categories of elements have the same form and are very diffi-

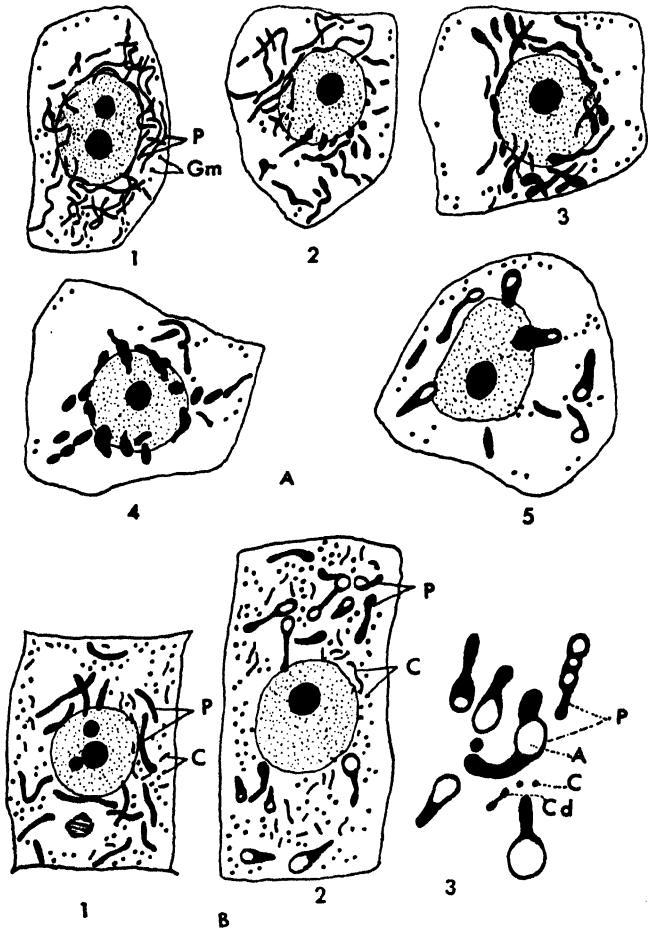


FIG. 56. — A. Development of chloroplasts in aerial root of *Chlorophytum Sternbergianum* (After MEVES). 1, meristematic cell, the chondriosomes represented by chondrioconts (*P*) only, the granules (*Gm*) MEVES believes to be metabolic products; 2-5, successive stages in the transformation of chondrioconts into chloroplasts, granules unchanged. B. Development of amyloplasts in pea root (After MOTTIER). 1, meristem, amyloplasts (*P*) shaped like chondrioconts, mixed with small, filamentous or granular elements (*C*) the only elements which MOTTIER believes to be chondriosomes; 2, mature root cell, amyloplasts (*P*) forming starch, the chondriome (*C*) unchanged; 3, detail, amyloplasts (*P*) forming starch (*A*), division figure of chondriosome (*C*) at *Cd*.

cult to differentiate, still the plastids would always be recognizable because of their slightly greater size. The two classes of elements correspond to permanent components of the cell, incapable of arising *de novo* and multiplying only by division. Nevertheless this American investigator considers them as *radically* different forma-

tions, but without, however, bringing forth the slightest histochemical proof in favor of this idea. Later (1921) he seems to have abandoned his opinion. He states that chondriosomes can not always be distinguished from plastids in meristematic cells of plants and seems to admit that the chondriosomes are plastids whose functions are multiple, the plastids of chlorophyll-bearing plants being only a special variety.

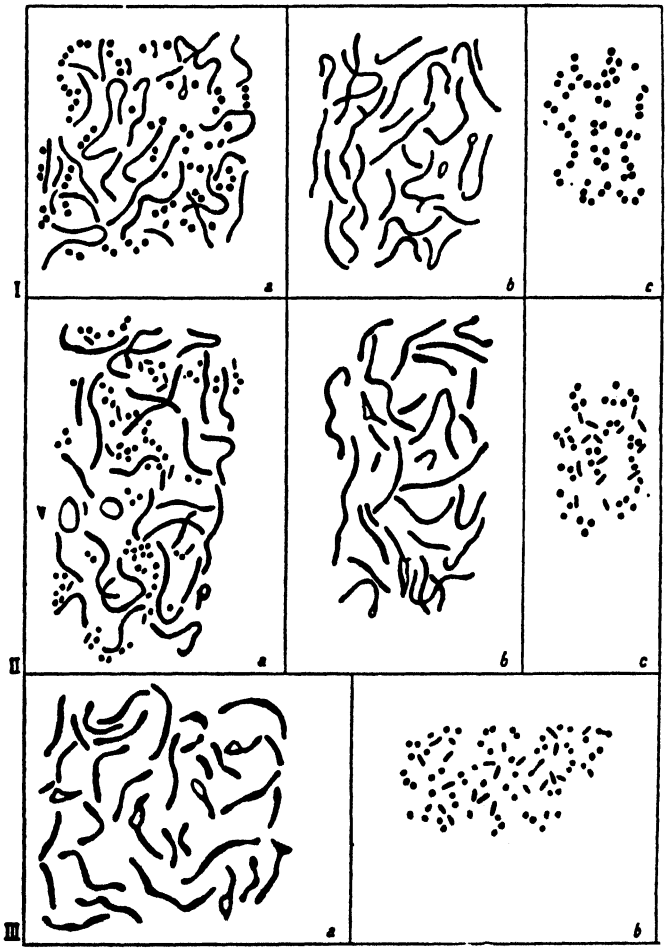


FIG. 57. — Chondriosomes and plastids in leaf cells of *Elodea canadensis*, I, in embryonic cells, II, at the beginning of differentiation. *a*, the chondriome; *b*, plastids and *c*, chondriosomes drawn separately. III, later stage. *a*, plastids; *b*, chondriosomes.

On the basis of research carried out exclusively in the phanerogams, MEVES (1918) expressed a theory which is the exact opposite of that held by most of the authors just discussed. This eminent cytologist observed that, in the meristem of buds, mitochondrial technique brings out both chondrioconts and granules, and that all the chondrioconts are transformed into chloroplasts in mature cells,

whereas the granules persist after the differentiation of the chloroplasts. He considers that only the chondrioconts correspond to the chondriosomes and the granules are not mitochondria but simply metaplasmic granules. Thus, according to MEVES, all the chondriosomes are transformed into plastids in the mature cells of the

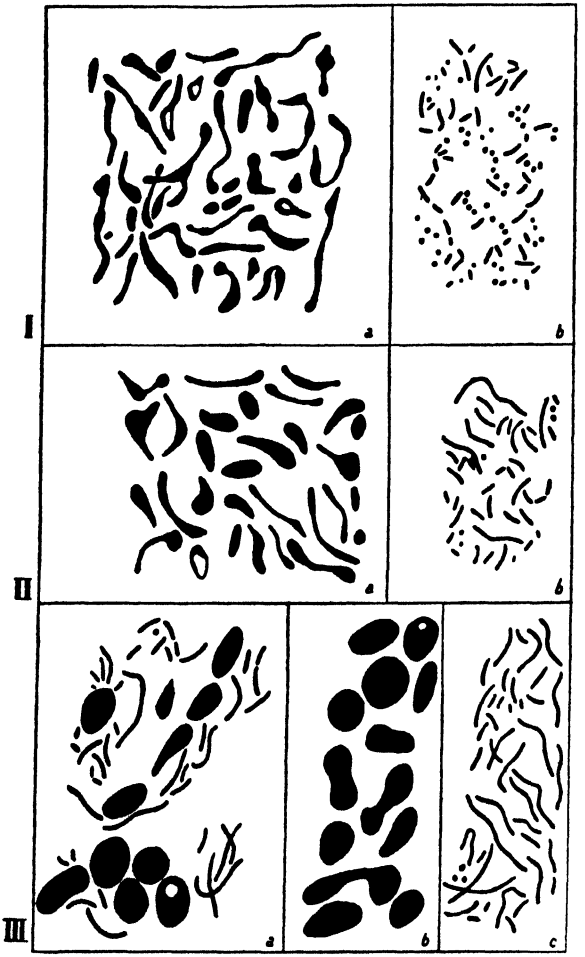


FIG. 58. — *Elodea canadensis* (fig. 57 cont.). I, II, further differentiation. *a*, plastids; *b*, chondriosomes changing shape. III, in a differentiated cell. *a*, the chondriome; *b*, plastids and *c*, chondriosomes.

phanerogams and it is those elements considered by most authors to be plastids, which MEVES believes are represented by the chondriosomes, whereas those described by others as chondriosomes have nothing in common with them.

BOWEN (1926-1929) at first adopted the theory of MEVES and considered the plastids as corresponding to the chondriosomes of animal cells. In later research he was led to modify his opinion and to admit the existence in the phanerogams of two categories of

elements which are different, but whose form is similar: the plastids, peculiar to chlorophyll-bearing plant cells; and other elements, which, hesitating for some unknown reason to liken to the chondriosomes of animal cells, he groups into a category which he calls the pseudo-chondriome.

WEIER (1930-1933), having obtained impregnation of the chloroplasts of *Polytrichum commune* by Golgi technique, felt justified in likening the plastids of plant cells to the Golgi apparatus described in animal cells. This theory was adopted by DUBOSCQ and GRASSÉ who, in a recent treatise, maintain that in animal cells there are two sorts of permanent, closely allied constituents of a lipoprotein nature, namely, the chondriosomes and the Golgi material, or dictyosomes, the latter being comparable to the plastids of chlorophyll-bearing plants.

Finally, KIYOHARA (1936), after making observations of living material carried out under improper conditions, thought he noticed that all the plastids normally appear as vesicles and that it is the mitochondrial technique which alters them and makes them appear as chondrioconts. But this Japanese investigator obtained plastids of vesicular form by osmic impregnation, the technique used for the detection of the Golgi apparatus. He thinks that chondriosomes do not exist in plant cells and that all forms described under that name correspond to images brought about by alterations in the plastids. He reports, however, that in mature cells there always exist, as well as the large vesicular plastids, other much smaller vesicles, but these he believes to be plastids in the act of degenerating.

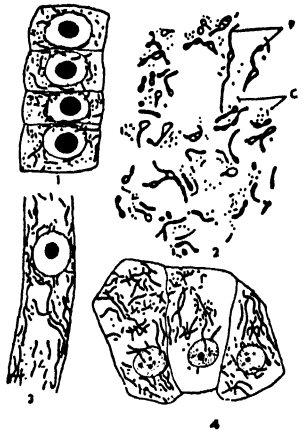


FIG. 59. — The chondriome. 1, 2, Root of *Cucurbita Pepo*. 1, meristem; 2, from differentiated cell of parenchyma, some thicker chondrioconts (*P*) form starch, short rods and mitochondria not perceptibly changed; a few elongate to thin chondrioconts. 3, young ascus of *Pustularia vesiculosa*. 4, frog's liver.

All these theories, aside from being essentially contradictory are, unfortunately, at variance with the facts. They are the result of hasty generalizations, founded on observations limited to certain types of cells and carried out, most often, with defective techniques. They give evidence of an insufficient knowledge of that which in animal cells has been designated as chondriosomes and which have been recognized in all the fungi.

It is now demonstrated by our research that in mature cells the plastids without chlorophyll generally keep the shape characteristic of the chondriocont. For example, it is in this form that they appear in the epidermal cells which we have already described (*Iris*, tulip, *Allium Cepa*). It is therefore impossible to attribute, as do MEYER and SAPĚHIN, the form of chondrioconts to division figures of plastids which have for the moment stopped dividing. It has also been proved in the most evident fashion, by our re-

search (1912-1923), as well as by that of FRIEDRICHS (1923), that the meristem of the bud of *Elodea canadensis* does not contain chloroplasts. This is contrary to the findings of NOACK, whose error can only be explained by supposing that in his preparations he confused tissues already differentiated and containing chlorophyll, with the meristem. It has been proved, besides, that among the elements which constitute the chondriome of meristematic cells in the phanerogams, it is impossible to distinguish those which will later become plastids, from those which will remain chondriosomes. Both have the same shapes and histochemical characteristics. Cytological work on animals (LEVI), as we have said, has established the fact that the chondriosomes are indeed permanent and clearly characterized elements of the cytoplasm. This is demonstrated, furthermore, by the research that we have done on the Saprolegniaceae, in which the absence of plastids makes this study more easy than in chlorophyll-bearing plants, and in which we have been able to follow the chondriome in living material during the entire development of these fungi. It is therefore not possible, for the time being, to consider the chondriosomes as dissimilar elements, or as products of cellular metabolism (*Allinantes*). The theory of ARTHUR MEYER, who labels the chondriosomes *Allinantes*, has never been verified, and is today definitely invalidated.

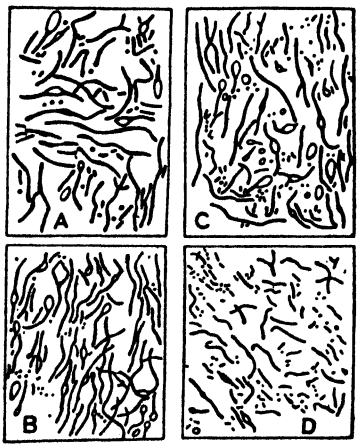


FIG. 60. — Similarity of the chondriome in A, the basidium of *Agaricus campestris;* B, the ascus of *Pustularia vesiculosa;* C, frog's liver; D, frog's kidney. Regaud's method (C, D, drawn from preparations of Professor POLICARD).

It has not been confirmed, either, that the plastids and the chondriosomes of embryonic cells of phanerogams can be differentiated by their dimensions, as was thought by MOTTIER who, without doubt, observed only cells already in the process of differentiation. Furthermore, our research has shown that the chondriosomes which are not transformed into plastids are in no wise exclusively shaped as granules, as MEVES believed. It sometimes happens, on the contrary, that in embryonic cells, the chondriosomes exist as chondrioconts, whereas the plastids are represented by mitochondria. This is so much the case, that when the chondriosomes appear as granules in meristematic cells, they almost always have the appearance of typical chondrioconts after the differentiation of the plastids has taken place, as in the leaves of *Elodea canadensis*. Therefore they are obviously chondriosomes. (Figs. 57, 58).

There is no basis, on the other hand, for the opinion of WEIER, for if it is true that the plastids are blackened by osmic impregnation because of their lipide constitution, it is also true that the

chondriosomes coexistent with them behave in the same manner. In the fungi, especially in the Saprolegniaceae, where there are no plastids, it is easy to demonstrate that the chondriosomes are intensely blackened by osmic impregnations, just as are the plastids of chlorophyll-bearing plants. Furthermore, the Golgi material in animal cells may be distinguished from the plastids by the fact that it does not stain with mitochondrial techniques.

The opinion of KIYOHARA results from an initial error of observing living material under defective conditions. This author leaves out of consideration that characteristic property of chondriosomes of becoming vesiculate during alteration (cavulation). He observed cells in which the chondrioconts were already transformed into vesicles and misinterpreted this phenomenon, mistaking the vesicles for normal shapes of the plastids and the chondrioconts for their altered shapes.

Chapter X

DUALITY OF THE CHONDRIOME

The first data obtained in our laboratory by EMBERGER and MANGENOT on the pteridophytes and the algae as well as the very meticulous study of the development of the chondriome in certain phanerogams, notably in the bud of *Elodea canadensis* and in the root of *Cucurbita Pepo,* led us, as early as 1920, to formulate a new theory which removes all the difficulties and accords with all the facts drawn from the development of plastids in the plant kingdom.

It has been seen that among the elements which constitute the chondriome in meristematic cells of the bud of *Elodea canadensis,* it is possible to distinguish between the chondrioconts which become chloroplasts during cellular differentiation, and the granular mitochondria which do not participate in this phenomenon but elongate into rods and later into chondrioconts. Now it has been known for a long time that chloroplasts have the ability to divide. Our work has shown that it is only by this process that they increase in numbers and that in differentiated cells the chondriosomes which persist along with the plastids are incapable of becoming chloroplasts. This shows therefore that the two categories of elements which constitute the chondriome of cells of the meristem develop separately and seem independent, one of the other.

The study of the development in the chondriome in the root of *Cucurbita* (Figs. 59, 61) will furnish a similar example. Here again, there is observed in the cells of the meristem a chondriome composed of two categories of elements: chondrioconts and mitochondria or short rods. If, in the course of cellular differentiation, the development of these elements is followed, it is seen that in the central cylinder, where elaboration of starch is not very active, the appearance of the chondriome does not change and one witnesses the production of small starch grains only within the chondrioconts. In the cortex, on the contrary, the chondrioconts undergo a considerable thickening and may be subdivided into rods or granules. The other chondriosomes keep their original size but sometimes take the form of chondrioconts which differ from the other chondrioconts by being thin. Our first impression is that there exist at this moment in the cells, two categories of chondriosomes, the one consisting of large chondriosomes, the other of little ones. The large chondriosomes represent the amyloplasts. At certain periods they are the seat of active elaboration of compound starch. Once having reached maturity, the starch grain formed in the interior of the larger chondriosomes considerably modifies the appearance of the latter. The starch is seen as a large grain surrounded by a thin mitochondrial layer. This layer is often prolonged as a tail, the

remnant of the elaborating chondriocont. When the starch is utilized, its absorption takes place within the amyloplast. The grain diminishes in volume while the outer mitochondrial layer grows and regenerates a chondriocont which will function again later.

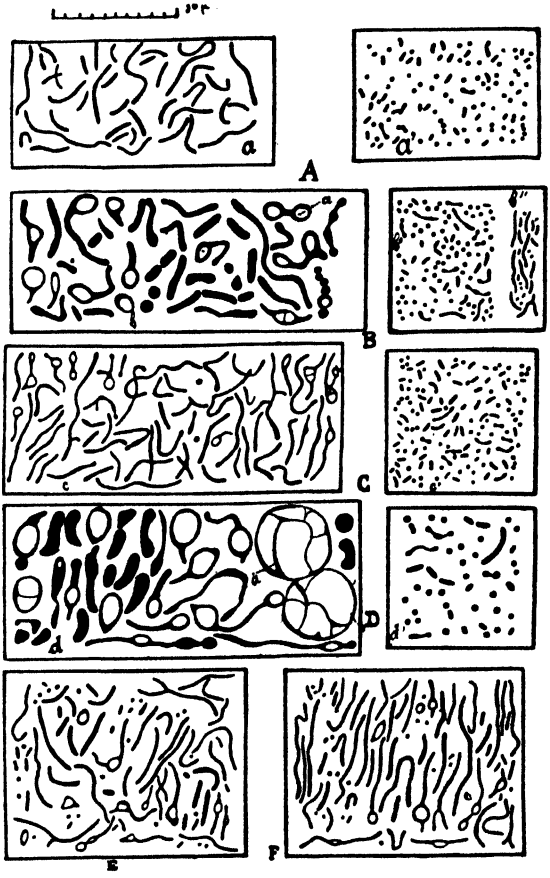


FIG. 61. — Chondriome. A-D, Root of *Cucurbita Pepo*. A, meristem; *a*, amyloplasts; *a'*, inactive mitochondria. B, cortical parenchyma; *a*, starch; *b*, amyloplasts; *b'*, inactive mitochondria, in some cases (*b''*) appearing like typical chondrioconts. C, parenchyma of central cylinder; *a*, starch; *c*, amyloplasts; *c'*, inactive mitochondria. D, cortical parenchyma of hypocotyl; *a*, compound starch grain in chloroplast, *d*; *d'*, inactive mitochondria. E, liver of frog. F, basidium of *Psalliota campestris*. In E and F, the mitochondria form vesicles of unknown significance. Regaud's method.

The chondrioconts, therefore, are not destroyed during elaboration and it is always the same elements which function in the formation of starch. Here again there are found the two categories of elements observed in *Elodea canadensis* and their shape generally makes it possible to follow them separately during their entire development. (Figs. 57, 58).

But these are rare and almost diagrammatic examples. In most cases, it is absolutely impossible among the elements which constitute the chondriome in cells of the meristem to distinguish those which will become plastids from those which will remain inactive,

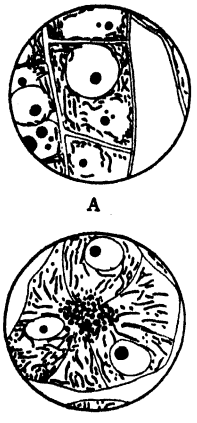


FIG. 62 (left). — Chondriome, showing morphological similarity of the meristem of pea root (A) and of pancreas cells of guinea pig (B), each fixed by Regaud's method and stained with iron haematoxylin. A, plastids and chondriosomes indistinguishable; B, chondriosomes and Claude Bernard granules. (After COWDRY).

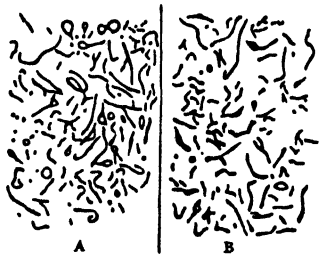


FIG. 63 (right). — Detail of chondriosomes (A) in pea root and (B) in mouse pancreas. × 1687. (After COWDRY).

for they are of similar form. Although in most cases it is the chondrioconts which become plastids, there are numerous exceptions, and cases are found in which the granules, as well as the chondrioconts, become plastids. It may even happen in some cases that the granules alone form the plastids, whether the other elements present have the form of chondrioconts or whether they also have the form of mitochondria. In the tuber of potato, for example, only granular mitochondria are found (Fig. 39) and it is through the agency of some of these that starch is elaborated. Furthermore, the distinction which we have made between the two categories of elements, *i.e.*, chondrioconts and granules, in the bud of *Elodea canadensis* and in the root of *Cucurbita Pepo*, is far from being general. Whether we can differentiate between the two categories depends, in *Elodea*, upon the state of activity of the bud and the period at which it was collected. There are buds in which this distinction is much less clear, others where it can no longer be made. The difference in size which we have noticed between the starch-forming plastids and the chondriosomes in parenchyma cells of the pumpkin root may itself diminish or disappear. For example, in cells in which the starch grains have just been digested, the chondrioconts which elaborated them, grow thinner and are indistinguishable from the other elements of the chondriome. Cells in the meristem of *Elodea* and of the pumpkin are derived from other embryonic cells in which the two categories are not distinguishable.

Theory of the author:- From the series of facts which we have just related, however, there arises the idea that the chondriosomes of cells of the meristem do not all have the same significance, although morphologically and histochemically similar. One is led to think that the chondriome of embryonic cells in phanerogams, although appearing homogeneous, is composed of two categories of chondriosomes, maintaining their individuality throughout cellular development. One of these categories corresponds to the *plastids* and may take on much larger dimensions during the course of development, by virtue of its active elaborative power. The other of these categories, which kept its original size after the plastids had become differentiated and to which we have provisionally given the name *inactive chondriosomes,* seems to have functions which are as yet not definitely determined.

FIG. 64 (left). — Chondriome in (1-4) differentiated colorless root parenchyma of *Athyrium Filix-femina* and in (5, 6) frog's liver. Regaud's method. (After MANGENOT and EMBERGER).

FIG. 65 (right). — Detail of chondriome in (A) *Saprolegnia* and in (B) epidermis of tulip perianth. × 3000. Regaud's method.

These two categories have the same shape in the phanerogams and it is almost always impossible to tell them apart in the meristems and usually, also, even in mature cells which do not have chlorophyll. They are, however, always perfectly distinct in some lower plants (bryophytes, algae) in which chlorophyll is present in all stages of development.

Thus considered, the plastids are not differentiated chondriosomes; they are a special type of chondriosomes. In fact, when the life history of the chondriosomes is followed in the phanerogams, one is struck by the chondriosomal characteristics which the plastids always maintain. The amyloplasts are usually typical chondriosomes and are only occasionally a little thicker than the other elements of the chondriome. They are not actually distinguishable from the inactive chondriosomes coexistent with them, until they become chloroplasts. In this case they appear as thickened bodies which, in the last analysis, are only hypertrophied chondriosomes containing chlorophyll. As for the variations in plastidial shape, we do not yet know whether they are caused by a growth of these elements or merely by imbibition.

Figure 60 gives a very exact idea of these facts. Here, in the pumpkin seedling, are shown side by side all the forms assumed by the two categories of elements in the course of cellular differentiation. It is seen that the two types of chondriosomes have the same shape through all the stages of cellular development—granules, rods, filaments—but these shapes are not always identical for both types at a given stage in development and there are stages in which one type appears as granules and the other as filaments. This makes it possible to distinguish them at every stage. Further-

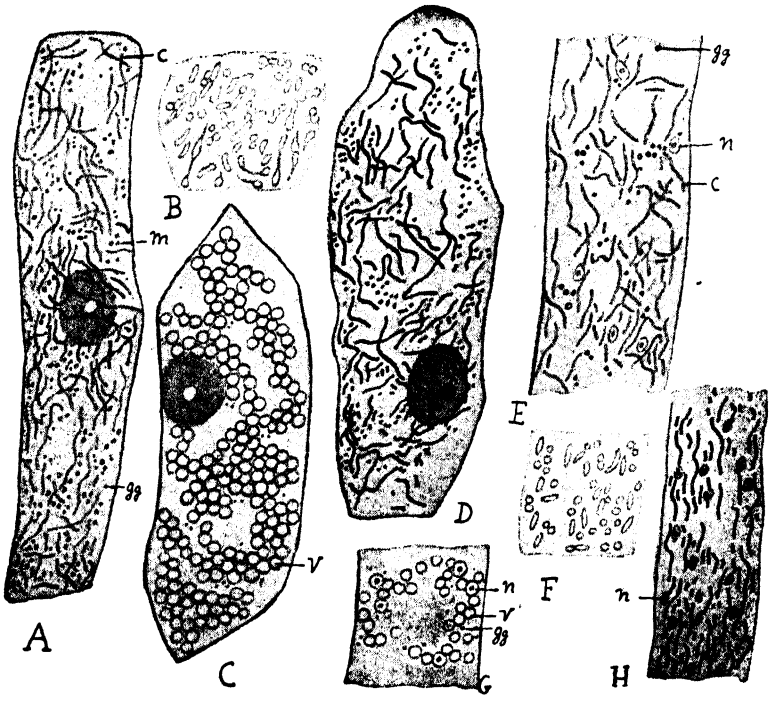


FIG. 66. — A-D, Epidermal cells of petal of tulip. *c*, leucoplasts; *m*, chondriosomes; *gg*, lipide granules. B, beginning of change in leucoplasts. C, vesiculation. D, cells fixed by Regaud's method. E-H, filament of *Saprolegnia*. *n*, nucleus; *c*, chondriocont. F, beginning of change in chondriosomes. G, vesiculation. *v*, vesicle. H, filament fixed by Regaud's method.

more, the two categories of elements are capable of division and frequent stages in division are observed.

If these two categories of elements are compared with the chondrioconts in liver cells of the frog or with those in fungi, as represented in Figures 60 and 61 (*cf.* also pp. 85 and 113), it is seen in a general way that it is the plastids which most resemble the animal chondriosomes and those of the fungi.

In a general way also, the inactive chondriosomes are a little smaller than animal chondriosomes and are less frequently found as chondrioconts. The plastids in general have the same dimensions as the chondriosomes of animal cells but in certain phases become much more voluminous.

One may object to the above theory on the ground that the two categories of elements do not have the same origin, that they develop separately and do not possess the same functions. This seems to imply that they are of different nature and that they correspond to radically different formations. This would bring us back to the first opinion of MOTTIER (1918). We shall see, indeed, that in cytology one must be suspicious of analogies in shape, since elements as different as young vacuoles and chondrioconts may show in certain phases of cellular development entirely analogous forms. These forms, moreover, are the forms of chromosomes as well. The histochemical point of view must therefore be the deciding factor.

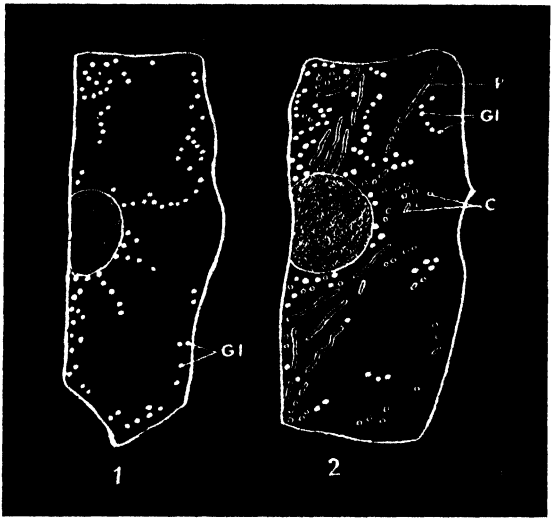


FIG. 67. — Epidermal cells of bulb scale of *Allium Cepa*, as seen under the ultramicroscope. 1, lipide granules (*Gl*). 2, granules, and the faintly luminous contours of chondriosomes (*C*) and plastids (*P*).

We shall see, however, that this objection is not valid here. Even before it was known that the chondriome of embryonic cells of the phanerogams was composed of two types of different elements, COWDRY (1918) made a meticulous comparison between the morphological and histochemical characteristics of the chondriosomes of pancreatic cells of the guinea-pig and those of the pea root. In the latter, the two categories are blended in the meristem and were not differentiated by the author. COWDRY concluded that the chondriosomes are identical in the two cases, including, of course, those which are transformed into plastids. (Figs. 62, 63).

Similar comparisons (Fig. 64) were undertaken by EMBERGER and MANGENOT (1920) between the chondriosomes of fern roots (including the amyloplasts) and those of various organs of the frog (kidney and liver). These observations led to the same results.

Histochemical and histophysical characteristics of chondriosomes and plastids:- We proceeded ourselves to make a comparative histochemical study of the two chondriosomal categories in chlorophyll-containing plants with respect to the chondriosomes of the Saprolegniaceae. In the latter, there exists only one category of chondriosomes which can be unquestionably homologized with the chondriosomes of animal cells as we have seen earlier in these pages.

This study consisted first of a comparative examination, made as accurately as possible, of the chondriome of epidermal cells from portions of a tulip perianth (white variety) and of the chondriome of *Saprolegnia,* both of which are particularly favorable for observation of living material. (Figs. 65, 66).

The living chondriome in these two different tissues has a similar morphological appearance and refractivity. It is represented in the tulip by long, thin, undulating and sometimes branching, chondrioconts which correspond to the plastids, and by the inactive chondriosomes in the form of granules or short rods. In *Saprolegnia,* except for the region at the tips of the hyphae, the chondriome, as we have seen, is formed exclusively of long and sometimes branched chondrioconts entirely similar to the tulip plastids. MEYER, who had observed them before the discovery of chondriosomes in the fungi of the same group, did not hesitate to liken them to plastids. These elements arise from mitochondria by growth and elongation, just as do the tulip plastids. It is in the mitochondrial form that they appear in the extremities of the hyphae of *Saprolegnia* and in epidermal cells in perianth parts of very young tulip flowers.

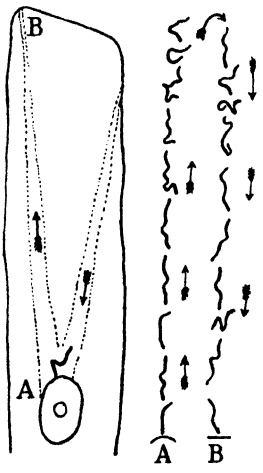


FIG. 68. — Epidermal cell of *Iris germanica.* At right, modifications in form observed when a chondriocont moves as from (A) to (B) in the diagram at the left where arrows indicate direction of current.

This study was completed later by similar observations which we made on epidermal cells from the leaves of *Iris germanica* and especially on epidermal cells from the bulb scales of *Allium Cepa.* In these bulb scales, the plastids and the chondriosomes present the same forms. They are both composed of a mixture of mitochondria, short rods and chondrioçonts. As the plastids do not elaborate starch, it is very difficult to tell the two categories of elements apart. The plastids, however, are often recognizable because they are slightly thicker than the chondriosomes and are longer when they are in the chondriocont stage. We will therefore review briefly here the principal results which we have obtained from this comparative study and add those reported by other authors.

The two categories of chondriosomes, plastids and genuine chondriosomes (Fr. *chondriosomes proprement dits*), of epidermal

cells which we have studied and the chondriosomes of *Saprolegnia* show exactly the same refractivity. Slightly superior to that of cytoplasm, this refractivity, although very slight, still permits the chondriosomes to be adequately seen. Under the ultramicroscope the two categories of elements of epidermal cells and the chondriosomes of *Saprolegnia* are distinguishable only under very favorable conditions. When visible, they always have the same appearance and are seen only because of their very faintly luminous contours. With the Zeiss micropolychromar they are made to appear very clearly with a different color from that of the cytoplasm, green on a red background, for instance, or yellow on violet. Chondriosomes and plastids of epidermal cells, as well as the chondriosomes of *Saprolegnia,* behave like extremely delicate elements which the least change in osmotic equilibrium, or the least pressure on the cover glass of a preparation, suffices to change into vesicles (cavulation). In a hypertonic medium they keep changing shape as long as the cell is living but as soon as it dies, they become vesiculate (Fig. 67).

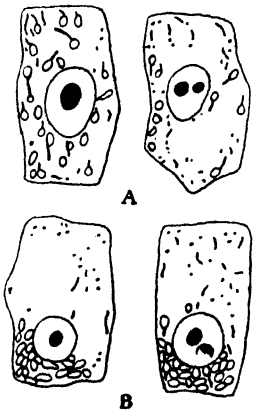


FIG. 69. — Cells from a tuber of *Ficaria ranunculoides* (A) before and (B) after centrifuging. A, chondriosomes with and without starch dispersed in the cytoplasm; B, at one side of the cell are found the nucleus and the starch-bearing chondriosomes; those without starch remain dispersed. (After MILOVIDOV).

We have already seen that in *Saprolegnia* the chondrioconts are moved about slowly by the cytoplasmic currents and that during these displacements they change shape, passing through the most varied forms. They are even able to branch by growing a kind of pseudopodium which afterwards is retracted. In epidermal cells of tulip in which cytoplasmic movements are very slow or do not exist, nothing of this sort is observed. In cells of *Iris germanica* and of *Allium Cepa,* however, which are very favorable objects for study, we have observed for the plastids these same displacements and the same instability of form. (Figs. 68, 70, 150). During the movement from place to place the plastids are capable of taking the most irregular shapes. They are capable of shortening by becoming thicker or of elongating by stretching out. They may form swellings along their long axes which are sometimes vesicular or they may put out transitory ramifications which are later retracted. Analogous observations have been made by EMBERGER for the leucoplasts in the epidermis of the bulb of *Asphodelus cerasiferus.* This proves that the plastids and chondriosomes are composed of a semifluid, very plastic substance, are in the same physical state and possess the same viscosity. During these observations, moreover, we could follow under the microscope, the process of division of the chondriosomes in the leaf cells of *Elodea canadensis,* that of the plastids in the epidermal cells of *Allium Cepa* and of the tulip, in which the displacements of the plastids and the cavulation (vesicu-

lation) which they undergo as they are altered can be observed (GUILLIERMOND, OBATON, GAUTHERET).

It may be added that MILOVIDOV and ORTIZ PICÓN have demonstrated that the chondriosomes and plastids have a specific weight rather like that of cytoplasm. After centrifuging, the plastids and chondriosomes remain scattered throughout the cytoplasm and it is only when the plastids contain starch grains that they are carried with the nucleus toward one extremity of the cell (Fig. 69).

Recent work of FAMIN has proved that, contrary to previous opinions (POLICARD and MANGENOT), the plastids of epidermal cells of tulip petals and the chondriosomes of *Saprolegnia* are both resistant to high temperatures. Although their visibility in living form is diminished and their chromaticity with mitochondrial techniques is lost, they are not destroyed by these high temperatures.

The leucoplasts and chondriosomes of epidermal cells of bulb scales of *Allium Cepa* and tulip petals behave as do the chondriosomes of *Saprolegnia* in regard to vital dyes. They stain selectively with Janus green, methyl violet 5B, Dahlia violet and a certain number of dyes recently mentioned (GUILLIERMOND and GAUTHERET). Employed in 0.0005-0.005% solutions, Janus green stains only the chondriosomes and leucoplasts, giving them a pale bluish green color in cells which are living and showing cytoplasmic currents. In 0.01-0.02% solution of the dye, the chondriosomes and leucoplasts are stained but in a manner clearly more accentuated in the former than in the latter and at the same time the dye accumulates in all the vacuoles which contain phenol compounds (oxyflavanol and tannin compounds). At higher concentrations it produces only sublethal staining with vesiculation of the chondriosomes and leucoplasts, resulting after a short while in the death of the cells.

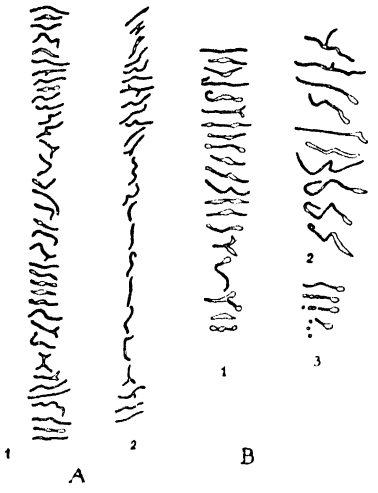


FIG. 70. — A, *Saprolegnia*. Forms taken by a chondriocont observed for half an hour. B, Epidermis of *Allium Cepa*. Forms taken by a leucoplast in half an hour.

The other dyes, which for the most part are very toxic, behave somewhat differently. In low concentrations (0.0002-0.0008%) they stain only the leucoplasts and the chondriosomes which are colored in exactly the same way. The cells remain living for a long time and show very active cytoplasmic currents. In solutions of 0.001% and above, the dyes accumulate at the same time in the vacuoles containing phenol compounds and finally stain the nucleus and cytoplasm to which they give a diffuse color in cells showing active cytoplasmic streaming, but in which they rapidly cause death. Staining is therefore sublethal.

As is seen, a single difference is shown between the chondriosomes and leucoplasts. Janus green stains the former more intensely than the latter with a 0.01-0.02% solution of the dye. In recent work, Miss SOROKIN has maintained that Janus green did not stain the leucoplasts. This is inexact, but it is certain that under some conditions Janus green stains the chondriosomes more intensely than the leucoplasts. The chondriosomes and plastids of epidermal cells as well as the chondriosomes of *Saprolegnia* are preserved with the reagent iodine-potassium iodide which makes them brown and renders them much more distinct than in living material. Both these elements of the chondriome are preserved with a 2% solution of osmic acid which does not turn them brown but, if the preparation is treated with pyrogallol after being in contact with osmic acid for half an hour or an hour, the chondriosomes and plastids appear gray. Both become even intensely black after being for a long time in a 40% solution of osmic acid (method of osmic impregnation used to reveal the Golgi material). Lastly, the chondriosomes and plastids of epidermal cells and the chondriosomes of *Saprolegnia* behave in exactly the same way in regard to fixatives. They are strongly modified and lose their chromaticity when treated with fixatives containing alcohol and acetic acid, and are preserved in their shapes and are stained clearly with all mitochondrial techniques (methods of REGAUD, BENDA, MEVES, HELLY, TUPA, VOLKONSKY, ALVARADO's modification of RIO-HORTEGA, etc.). They are stained by DIETRICH - SMITH's method (used for the detection of lecithins) and after sufficient time by indophenol blue. This behavior, together with that described above, proves that the chondriosomes and plastids have a similar lipide constitution.

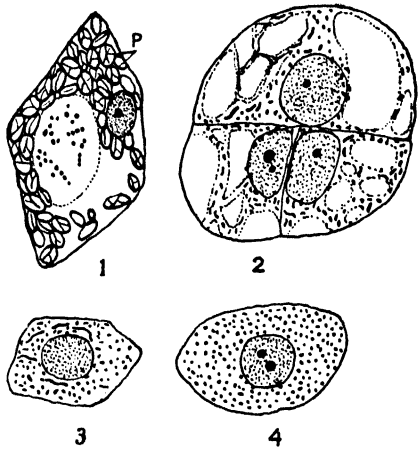
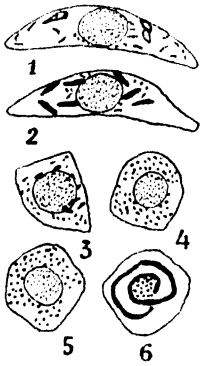


FIG. 71. — Chondriosomes and plastids in the prothallus of *Adiantum capillus-Veneris*. 1, vegetative cell; *P*, plastid. 3, very young egg. 4, mature egg. 2, young embryo after division of fertilized egg. Regaud's method. (After EMBERGER).

MILOVIDOV was able, moreover, to show in the chondriosomes and plastids all the protein reactions, just as GIROUD had demonstrated them for the chondriosomes of animal cells. The two categories of elements, therefore, have the same lipoprotein constitution. More recently, MILOVIDOV demonstrated that the chondriosomes and plastids in the roots of pea and of *Allium Cepa* do not give the Feulgen reaction and in consequence do not contain thymonucleic acid. Chloroplasts, nevertheless, offer much greater resistance to fixatives containing acetic acid and alcohol than the other plastids and the chondriosomes. Furthermore, in regard to

the vital stains for chondriosomes (Janus green, Dahlia violet, methyl violet, etc.) the chloroplasts do not behave like the leucoplasts, in that the chloroplasts are not stained as long as the cells are alive. On the contrary, as STRUGGER has shown, living chloroplasts are stained by rhodamine B which, at a sufficient concentration, gives them, with time, a very characteristic yellowish color in cells which show cytoplasmic currents and which remain alive for a very long time. (Rhodamine B also stains the chondriosomes and leucoplasts but very faintly.) The chloroplasts are distinguished from other plastids by their property of reducing silver nitrate. This property was noted first by MOLISCH and, as we have seen earlier, is manifested only in living cells. RUHLAND and WETZEL found it possible, with this reaction, to demonstrate the presence of chloroplasts in the chondriosomal state in generative cells of *Lupinus luteus* and of some other plants. GAVAUDAN in his



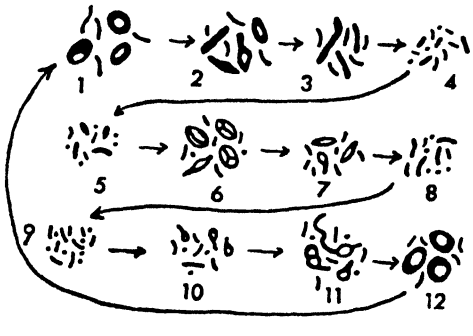


FIG. 72 (left). — Chondriome of antherozoids of *Adiantum capillus-Veneris*. 1, 2, antheridial initial. 3, 4, sperm mother cell. 5, 6, stages in the formation of the antherozoid. Regaud's method. (After EMBERGER).

FIG. 73 (right). — Behavior of the chondriome during the life cycle of a fern. 1, leaf; starch-forming chloroplasts and chondriosomes. 2-3, formation of spores; decreasing activity of plastids. 4, spore mother cells; inactive plastids indistinguishable from chondriosomes. 5, mature spore; plastids become active. 6, prothallus; starch-bearing chloroplasts. 7, 8, sexual cells; second cessation of activity of plastids. 9, egg; homogeneous chondriome. 10, developing embryo; certain chondriosomes secrete starch. 11, 12, adult plant; 11, amyloplasts of the root. 12, chloroplasts of the leaves. (After EMBERGER).

study of the hepatics claimed that this property is common to all plastids, even those lacking in chlorophyll, and considers it a means of distinguishing plastids from chondriosomes. But our later research, as well as that of GAUTHERET and of MIRIMANOFF, did not confirm this assertion and proved that plastids without chlorophyll do not reduce silver nitrate in living cells any more than do chondriosomes. This property which chloroplasts have of reducing silver nitrate in living cells has nothing in common with the black coloration of the plastids and chondriosomes in cells treated by ALVARADO's modification of RIO-HORTEGA's method, or with that sometimes taken on when they are impregnated with silver (Golgi method). It does, however, explain why PENSA found that only the chloroplasts were stained when he treated living tissue with Golgi's technique (silver impregnation).

All these facts lead us to the conclusion that the two categories of cytoplasmic organelles in chlorophyll-containing plants both show the characteristics of chondriosomes and it is evident that there is no criterion, unless it is the ability of the plastids to form starch and chlorophyll, for including the inactive chondriosomes rather than the plastids in the formations known in animal cells as chondriosomes. On the contrary, the plastids by their elongated chondriocontal forms sometimes resemble the chondriosomes of animals even more than do the inactive chondriosomes of plants.

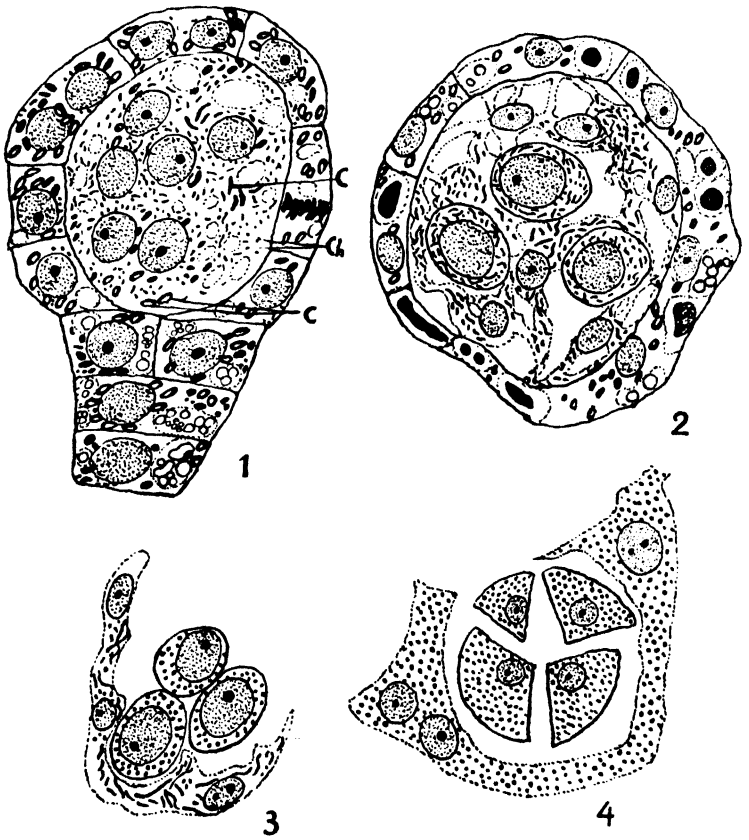


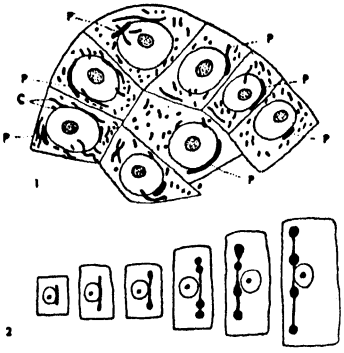
FIG. 74. — Fern sporangia. Successive stages in the return to a homogeneous chondriome by resorption of starch and loss of pigment in the chloroplasts. 1-3, *Asplenium Ruta-muraria*. 1, sporogenous cells; 2, spore mother cells; 3, tapetum and spore mother cells. 4, *Pteridium;* tetrad and tapetum. Regaud's method. (After EMBERGER).

The plastids are sometimes, however, slightly larger. Furthermore, the inactive chondriosomes unquestionably have the characteristics of chondriosomes from which it is impossible to separate them, as MEVES does, for they, too, show in a great number of cases the form of typical chondrioconts.

These two categories of elements, therefore, fit the definition of chondriosomes. They correspond to organelles seeming to be incapable of forming other than by division, they have the shape

of granules, rods or chondrioconts, are able to change from one of these forms to the next, and are characterized by a number of well determined physical and chemical properties. The theory of the duality of the chondriosomes has been remarkably confirmed by a study of the life history of the chondriome throughout the plant kingdom and is particularly well supported by the investigations of MANGENOT and EMBERGER.

Development of chondriosomes and plastids among the plant groups:- EMBERGER (1920-23), in investigating the pteridophytes (Figs. 71-74), found that the egg cell in the ferns contains a chondriome exactly like that in the animal cells, in which chondriome it is not possible to distinguish the plastids from other chondriosomes. However, in those prothallial cells from which the egg cell is derived there are both large chloroplasts and small chondriosomes. As these cells differentiate in the course of the formation of the egg, EMBERGER has shown that their chloroplasts lose chlorophyll and



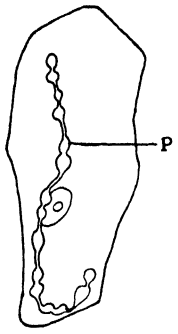


FIG. 75 (left). — *Selaginella Kraussiana*. 1, vegetative tip of the stem, each cell containing chondriosomes (*C*) and a slightly larger plastid (*P*) appressed to the nucleus. 2, Diagram of developing plastid during cellular differentiation. (After EMBERGER).

FIG. 76 (right). — *Selaginella Kraussiana*. Plastid (*P*) in living parenchyma cell of the stem. (After EMBERGER).

at the same time diminish progressively in volume, with the result that they take on, little by little, the appearance of small elements which it becomes impossible to distinguish from the chondriosomes. The same phenomena occur in the formation of the antherozoids. The fertilized egg resulting from the fusion of these cells shows, therefore, a homogeneous chondriome. In the embryo, some of the elements of this chondriome differentiate anew. In the leaves they become chloroplasts, in the stem and root they become large chondrioconts which represent the starch-forming plastids.

In the epidermal cells of leaves which will produce sporangia, both chloroplasts and inactive chondriosomes are encountered. In the young sporangia, however, the chloroplasts again lose their chlorophyll and appear as typical chondriosomes, indistinguishable from the inactive chondriosomes. From the time that the spore begins germination, these typical chondriosomes grow larger, become impregnated with chlorophyll and take on again the character of chloroplasts.

Analogous phenomena were found later (CHOLODNY, 1923) in the submerged leaves of *Salvinia natans* which, as is known, look like roots, as they have no chlorophyll and are reduced to veins. The chloroplasts which are present at first in these leaves lose their chlorophyll and take on the appearance of chondriosomes absolutely indistinguishable from the genuine chondriosomes.

Investigating the Selaginellas (Figs. 75, 76), EMBERGER observed that in cells of the meristem and in the spores where the chondriome contains above all long chondrioconts, there is only a single colorless plastid, which also appears as a chondriocont but is a little larger than the others and is pressed against the nucleus. This organelle, already pointed out by HABERLANDT, SAPĚHIN and P. A. DANGEARD, which is at first scarcely distinct from the other chondrioconts, grows little by little during cellular differentiation until in each cell of the leaf and stem there is a single chloroplast. It is composed of a series of large swellings united by thin filamentous portions which are brought about by uncompleted divisions of the initial plastid. This fact is particularly interesting from two points of view. First, in *Selaginella* and *Anthoceros* (in which there is also in each cell only one chloroplast, crescent-shaped in this case, more or less appressed to the nucleus but always larger than the small colorless plastids of embryonic cells of *Selaginella*), there are found the intermediate steps between the plastids of the phanerogams and the large chloroplasts of some algae. This shows that there is no reason to consider the chloroplasts of the algae as different from ordinary plastids. Secondly, the presence of this solitary plastid, which can be followed through all cells of *Selaginella* and which divides when the cell does, furnishes undeniable proof that the plastids maintain their individuality during the course of cellular development and arise always from the division of pre-existing plastids. The behavior of the plastids in the phanerogams makes this seem likely but does not sufficiently demonstrate it.

The investigations of MANGENOT (1922) brought out that the algae behave differently, from the point of view of plastidial development, depending on whether the chlorophyll persists in all stages of development or disappears in the sexual organs. When it persists, the plastids are distinguished from the chondriosomes in all stages of development, including the egg, by their size, their shapes and their colors. Consequently chloroplasts and chondriosomes are coexistent at all times.

MANGENOT demonstrated the presence of large chloroplasts and small chondriosomes at all stages in the Siphonales. These had already been encountered in *Vaucheria* by RUDOLPH. These two categories of organelles, in spite of the difference in their dimensions, have analogous shapes and divide at the same time in some phases of development. Such is also the case in the Fucaceae, in which, however, the chlorophyll and fucoxanthin lose their intensity in the oogonium and in the apical cells. There the phaeoplasts take on the form of small rod, or spindle-shaped

organelles, differing only slightly from the chondriosomes. Furthermore, the chlorophyll and fucoxanthin disappear in the mother cell of the antheridium and the phaeoplasts in this cell come to look like chondrioconts and are distributed among the antherozoids in such a way that each encloses a single phaeoplast. This plastid later is filled with a carotinoid pigment and becomes the stigma. The stigma, then, is simply a structure derived from a phaeoplast.

Plants in which the chlorophyll does not persist but rather disappears in the sex organs are found in the Rhodophyceae (Florideae) and the Characeae. In the Rhodophyceae, for example in *Lemanea,* the cells of the thallus (Fig. 78, I and the upper portion of A) enclose large, ribbon-shaped rhodoplasts, sometimes anasto-

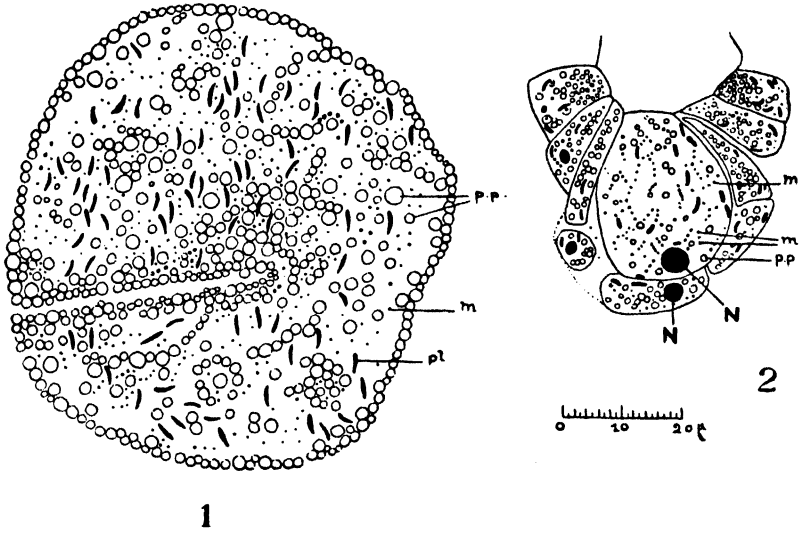


FIG. 77. — Fucus *vesiculosus.* Fusiform and rod-shaped plastids (*pl*) with mitochondria (*m*) and fucosan granules (*p.p.*) in *Fucus vesiculosus.* 1, apical cell; 2, two celled embryo. *N,* nucleus. Regaud's method. (After MANGENOT).

mosing to form a network, together with small chondriosomes. In those portions of the thallus containing little chlorophyll (Fig. 78, I_1 and lower portion of A), these elements grow thinner and appear somewhat like chondrioconts. In the rhizoids (Fig. 78, I_2), in which neither chlorophyll nor phycoerythrin exists, the plastids become very small and look so like the inactive chondriosomes that it becomes impossible to tell them apart. The trichogyne and other cells of the carpogonial branch (Fig. 78, A) develop from an ordinary cell of the thallus containing large rhodoplasts. A regression of chlorophyll and of phycoerythrin may be observed in these cells. The plastids lose their color and are transformed into small rods becoming like the chondriosomes which are present with them in the cell. Then the carpogonium shows a chondriome in which all distinction between plastids and chondriosomes is impossible. This chondriome persists in the first cells of the gominoblast fila-

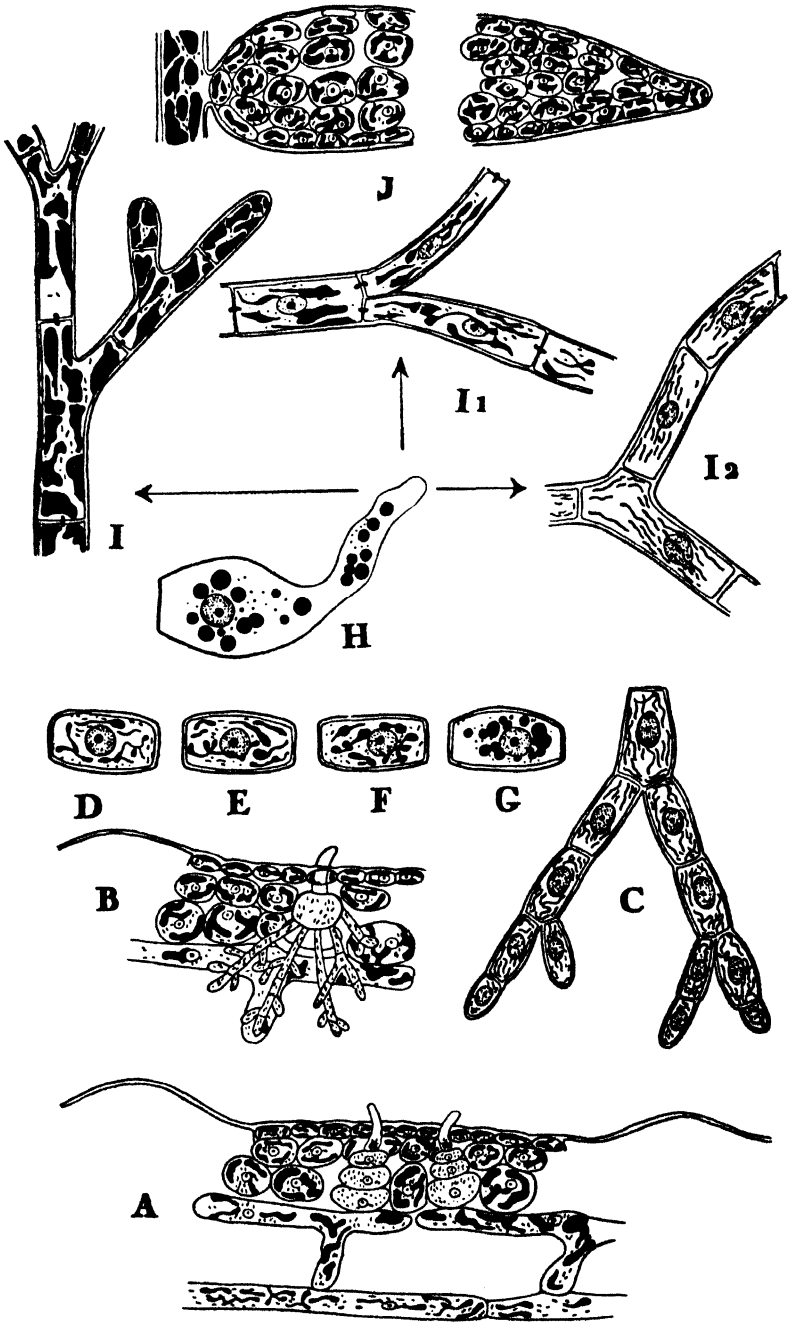


FIG. 78. — Rhodophyceae. Life history of the plastids in *Lemanea*. A, fragment of wall of cystocarp with two carpogonial filaments. B, Id. with gominoblast filaments. C, detail of filament. D-G, carpospores. H, germinating spore. I, assimilating filament. I_1, filament near substratum. I_2, rhizoid. J, cystocarp. (After MANGENOT).

ment (Fig. 78, B, C) and then there can be followed in these cells (Fig. 78, D-F) a differentiation of large rhodoplasts from some of the elements of the chondriome. In the mature carpospores (Fig. 78, G, H) there are found fairly large, well differentiated, disc-shaped rhodoplasts.

In the Characeae, MANGENOT found small chloroplasts and chondriosomes in the apical cells but in the oosphere there is no chlorophyll. In the cells which give rise to the oosphere, MANGENOT observed a regression of the small chloroplasts. They lose their chlorophyll and are transformed into mitochondria or short rods which can not be distinguished in the young oospheres from the inactive chondriosomes. In the course of development of the oosphere, some of the mitochondria and rods representing the former chloroplasts, elongate and take on the shape of typical chondrioconts, whereas the inactive chondriosomes persist in the form of mitochondria. The chondrioconts then elaborate numerous starch grains in the usual way. They correspond therefore to amyloplasts.

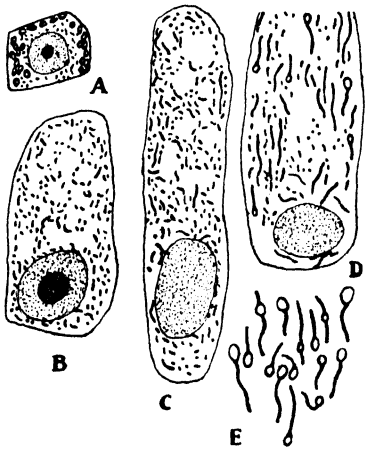


FIG. 79. — *Chara fragilis*. Development of the chondriome in the egg. A, young egg. B, beginning of differentiation. C, later stage, appearance of chondrioconts. D, mature egg; chondrioconts forming starch. E, detail of (D).

Information on the development of the plastids is still scarce in the bryophytes. It has already been seen that RUDOLPH, SCHERRER, SAPÉHIN, and MOTTIER believed that chlorophyll persists in these plants in all stages of development. They state that bryophytic cells always contain chloroplasts and chondriosomes at the same time. The fact is well demonstrated for *Anthoceros* but is questioned for the other bryophytes. Whereas P. A. DANGEARD, P. DANGEARD, GAVAUDAN and WEIER tend to confirm it, ALVARADO, SENJANINOVA, MOTTE and CHALAUD oppose it and believe that the chloroplasts are derived from the chondriosomes. ALVARADO has stated that in young paraphyses of *Mnium cuspidatum* there are no chloroplasts but only chondriosomes of which some afterwards become transformed into chloroplasts. According to MOTTE, most mosses contain in the apical cell of the stem both small lenticular chloroplasts and chondriosomes, while in some species (*Grimmia crinita*) only chondriosomes are found, a part of which develop into chloroplasts in cells arising by division from this apical cell. MOTTE described a regression of chloroplasts in the sperm mother cells. In the formation of these cells, the chloroplasts lose their chlorophyll and become transformed into long chondrioconts. These fragment to form granules that it is impossible to distinguish from the chondriosomes, which are coexistent with them. According to MOTTE,

the archegonium is formed from an initial cell containing only chondriosomes and he finds that the egg also lacks chloroplasts. These facts make it seem extremely probable that, at some stages in development in the bryophytes, the chloroplasts are capable of regression and of taking on mitochondrial form just as in the pteridophytes and in certain algae.

However this may be, it follows from data presented for the first time in the splendid work of EMBERGER and MANGENOT, that the chloroplasts may, under some conditions, lose their chlorophyll, become considerably smaller, and take on again the size and shape of typical chondriosomes. *The form typical of chondriosomes and the form typical of chloroplasts are therefore reversible* and the chloroplasts may be considered as chondriosomes containing chlorophyll. The chloroplast is derived from a chondriosome and may under certain conditions lose its chlorophyll and revert to the state of the chondriosome, the state which is characteristic of the functionally inactive phase of plastids, exactly as the amyloplast resumes its initial form after the absorption of its starch. If this reversibility of chloroplasts is not ordinarily observed in phanerogams, it is doubtless because, according to the research just discussed, the chlorophyll-containing tissues in these plants achieve a state of differentiation too advanced for a regression to take place such as occurs in plants less evolved.

Therefore the fact that chlorophyll is elaborated in a continuous or discontinuous manner influences very notably the appearance taken by the chondriome in chlorophyll-containing plants. In the first case, the cells contain constantly and at the same time, both large chloroplasts and small chondriosomes; in the second case, on the contrary, there are found, during the periods when chlorophyll is lacking, chondriosomes which all together constitute a chondriome analogous to that encountered in cells of animals and fungi, and in which it is not possible to distinguish the plastids from the genuine chondriosomes, and it is only during phases of elaboration of chlorophyll that some of the chondriomal elements grow and become large chloroplasts.

These facts will be illustrated by a study of saprophytic or parasitic phanerogams in which chlorophyll is formed only in very small quantities or, in some species, not at all. Among these, *Limodorum,* a saprophyte which is poor in chlorophyll, contains only very small chloroplasts. In the genus, *Orobanche,* in which chlorophyll has disappeared, plastids elaborating starch are still observable which in some portions of the plant also contain carotinoid pigments. In the stem of *Monotropa,* in which chlorophyll is also lacking, there is no longer production of starch, except in the endodermis, and in that region only, can the plastids be distinguished from the chondriosomes. This distinction is impossible in the tissues of *Cytinus Hypocistis,* a plant more completely adapted to parasitic life. This plant is also lacking in chlorophyll and has lost its power of forming starch (EMBERGER and MANGENOT, unpublished observations).

Considering this from a different angle, one of our students, GAUTHERET, has shown that the production of chlorophyll may be experimentally obtained in most roots when they are grown under certain conditions (in the presence of light and in media containing a certain quantity of sugar). Thus, for example, in the root of the barley, whose cells normally contain a chondriome composed of a mixture of chondrioconts, rods and mitochondria, in which plastids and genuine chondriosomes are indistinguishable, GAUTHERET has succeeded in obtaining large chloroplasts, entirely comparable to those encountered in the leaves of the same plant, by a differentiation of some of these elements.

The series of investigations undertaken with the aid of mitochondrial methods, either by us in the phanerogams or in our laboratory by EMBERGER and MANGENOT on the pteridophytes and algae, have permitted us definitively to solve this problem which has remained obscure for so long, namely, the origin and life history of chlorophyll-containing plastids. The progress made since the work of SCHIMPER and MEYER may be judged by comparing the state of this question when there were no methods for preserving plastids in stained preparation and when the investigator had to be content with incomplete observations of living material, to the present status of the question with its very accurate data obtained by mitochondrial technique, completed by observation of living material.

It is now evident that the chlorophyll-containing plants possess two categories of organelles which are permanently found in every cell, both of which show all the characteristics of chondriosomes in animal cells.

The first category, whose rôle it has not been possible to define completely, corresponds to the chondriosomes found in cells of animals and fungi. Its elements may be called *inactive chondriosomes* or even *genuine chondriosomes.*

The second category, peculiar to chlorophyll-containing plants, corresponds to the plastids of SCHIMPER. These are to be distinguished under the name *plastids.*

These two categories of organelles seem each to keep their individuality in the course of cellular development and seem to form only by division of pre-existing elements. This behavior, difficult to demonstrate for the plastids in the cells of phanerogams, is *absolutely proved* by what is known regarding the chloroplasts of green algae, by the study of *Anthoceros,* and especially, by the observations of EMBERGER on *Selaginella.* It is extremely probable, also, that these characteristics are shared by the genuine chondriosomes, since these elements are present in all cells, since they have never been observed to form *de novo* or to disappear and since they are capable of division. Indirect arguments in favor of this opinion may be drawn, moreover, from the behavior of the plastids which are very similar to them.

The two categories of elements have (except in many algae in which chlorophyll persists in all stages), the same characteris-

tic forms: granules, rods and filaments, capable of changing from one shape to the other. They offer, moreover, and this is much more important, the same histophysical characteristics (same refractivity, same viscosity, same process of alteration) and the same histochemical characteristics, even to their behavior with a great number of chemical reagents and dyes. For these reasons, it is generally impossible to tell them apart in embryonic cells of plants in which chlorophyll is not continuously elaborated. The plastids, therefore, are distinguished from other chondriosomes only by the fact that they are the centers of very active elaborations which considerably modify their shape. This is true in cells lacking in chlorophyll in which plastids elaborate starch, and especially true in green cells in which the plastids become voluminous because of the chlorophyll which they accumulate. Moreover, these modifications of form may be only transitory. The amyloplasts in cells without chlorophyll, as soon as the starch has been absorbed, go back to their forms of typical chondriosomes. The chloroplasts themselves may in some cases lose their chlorophyll and return to their original state as chondriosomes. In a word, *the chondriosomal form is the form taken by these organelles during the functionally inactive period.* The only distinction, therefore, that exists between the animal and fungal cell on the one hand, and the cell of the green plant on the other, is the presence of plastids, the second category of chondriosomal elements. This distinction is related to the existence of the chlorophyll function which characterizes green plants.

These facts, now exactly demonstrated by our work and that of our students, carried on over a period of thirty years, have led us to formulate the theory of the duality of the chondriome in chlorophyll-containing plants. This consists in stating that the chondriosomes of chlorophyll-containing plants are composed of two categories having between them the same relationships which the heterochromosomes bear to the autochromosomes, the first category (the *genuine chondriosomes*) being very similar to the chondriosomes of animals and fungi, the second category (the *plastids*) composed of a supplementary line of chondriosomes related to photosynthesis, which characterizes these plants. It is obvious that the two categories must, after all, possess differences in chemical constitution. Otherwise it could not be explained why one has functions which the other does not have. Nevertheless these differences, probably very slight, do not appear during histochemical analysis. In any case, both categories of elements have very *closely allied lipoprotein constitutions* and form in the cytoplasm a disperse lipoprotein phase. They are differentiated only by one of them manifesting a function which is lacking in the other.

The question is therefore definitely solved as far as the facts are concerned and it is demonstrated that the chondriosomes and plastids are two individual cellular components with the same lipoprotein constitution, capable of presenting identical shapes but

developing side by side without any genetic bond between them.

This is undeniable and cytologists are more and more inclined today to admit it. There is only one point which still remains hypothetical and this is the identification of plastids with chondriosomes which many cytologists still refuse to accept. In order to settle the question definitely we should have to know more precisely the chemical constitution of these two categories of elements and this is impossible in the present state of science. We should also have to obtain information on the phylogeny of plastids and chondriosomes which now escapes us. That which is certain is that the chondriosomes and plastids can only be regarded as very *closely allied formations.* It is illogical to deny these incontestable relationships as so many cytologists still do, for it is a much greater assumption to consider them as essentially different formations than it is to put them both together under the heading of chondriosomes. Also, our theory, which nothing has contradicted since we formulated it nearly twenty-five years ago, seems to be the only interpretation possible in the present state of knowledge. It has, furthermore, the advantage of suggesting a series of working hypotheses, not only with respect to the rôle of the genuine chondriosomes which is at present almost completely unknown, but also with respect to the physiological functionings of the plastids in the elaboration of chlorophyll, their functioning in photosynthesis and in the condensation of hexoses into starch. It is possible to imagine that a day will come when the physico-chemical study of the cytoplasm will show us that the chondriosomes have a very general function of which that manifested by the plastids is only one specific part.

Phylogenesis of chondriosomes and plastids:- Nothing is known about the phylogenesis of these two categories, chondriosomes and plastids, whose chemical constitutions are so closely allied. The fungi, which many botanists consider to be derived from the algae, show, however, no trace whatever of plastids. There is only one line of chondriosomes in them. In the Cyanophyceae (p. 41), which by reason of the primitive structure of their nucleus, may be considered as the most inferior algae known, it is impossible, as we have seen above, to detect the presence of chondriosomes and of plastids. The chlorophyll is diffuse in the cytoplasm of these algae. It has sometimes been thought that the lipoprotein substance of plastids and chondriosomes was also diffused in the cytoplasm. This, however, is only an hypothesis based on the absence of plastids and on the fact that chlorophyll can hardly have as substratum any other than a lipoprotein substance. In all flagellate algae, another inferior group thought to be the common ancestors of algae and protozoans, there exist very varied forms, some with chlorophyll, some without. All contain chondriosomes (CHADEFAUD). We have seen that in chlorophyll-containing forms the plastids sometimes look like the chloroplasts of phanerogams (Euglenas, Peridiniaceae), sometimes appear as a single voluminous

chloroplast (certain Chrysomonadales). According to CHADEFAUD, the forms possessing only a single chloroplast represent the most primitive types.

Among the forms without chlorophyll, VOLKONSKY has reported in *Polytoma uvella* the existence of a single leucoplast per cell, which appears as a fine network spread throughout the cytoplasm. This leucoplast elaborates starch. More recently Miss RABINOVITCH found the same organelle in *Polytomella coeca.* It has been possible to suppress the chlorophyll by various cultural processes in the Euglenas but it has not been possible previously to understand what became of the chloroplasts in the forms deprived of chlorophyll. In the phylogenetic series, above the flagellated algae are placed the green algae, such as the Chlorophyceae, which often possess only a single voluminous chloroplast. We then progress through the Rhodophyceae, the Characeae and bryophytes to the pteridophytes and phanerogams, in which the plastids are always fragmented into numerous small elements similar to chondriosomes.

Chapter XI

HYPOTHESES RELATIVE TO THE ROLE OF CHONDRIOSOMES AND PLASTIDS

It has just been seen that cytological investigations, carried out during recent years, have shown that the cytoplasm always contains in suspension various inclusions, among which the most important are the chondriosomes to which, in chlorophyll-containing plants, are added the plastids. The latter present a close analogy to the chondriosomes but may be considered as a line distinct from these elements.

Immediately, this raised the question as to the rôle of the chondriosomes and plastids. We find ourselves here on uncertain ground, for it must be recognized that if, at the present moment, our morphological knowledge is very advanced, we are still extremely ill-informed as to the rôle which must be attributed to these various elements in the functioning of the cell. As DEVAUX says, "The fact is that we do not sufficiently know the inner organization of the cell, for all that a microscopic study makes possible is a first approximation, manifestly incomplete. We should, for complete knowledge of the cellular mechanism, be able to reach the molecules themselves, that is, the elementary particles of the cell, and study them from the triple point of view of structure, of molecular attractions and movements, as well as from the point of view of reciprocal relations." Perhaps some day the progress of physical chemistry will give us precise information on this point, but for the moment we must be content with hypotheses which are still very vague and which we will take up here as briefly as possible. Investigations at the beginning of the study of chondriosomes led various authors, REGAUD in particular, to attribute to the chondriosomes of animal cells, an important rôle in the phenomena of secretion. According to this opinion the chondriosomes are organelles through whose agency are elaborated very diverse products of cellular activity: zymogen granules, fats, pigments, *i.e.*, the chondriosomes have a rôle exactly like that of the plastids in chlorophyll-containing plants. Thus the chondriome appears as the secretion apparatus of the cell. Although the investigations in plant cytology have demonstrated the very curious fact that it is precisely the plastids of chlorophyll-containing plants which show exactly the same forms and the same histochemical reactions as the chondriosomes, we have seen, however, that more accurate research on animal cells and on the thallus of fungi, employing the control methods of direct observation of living material and of vital staining, have not been able to confirm this secretory rôle except in exceptional cases (NOËL). The direct participation of chondriosomes in phenomena of secretion seems to us therefore to be hypothetical and in reality we know nothing as to

the rôle of these organelles. Nevertheless, the fact that the chondriosomes usually show no morphological evidences which can be related to their participation in secretory phenomena does not exclude the possibility that they may have a rôle in these phenomena, and it is possible that later studies may succeed in demonstrating this rôle which is suggested by the close parentage of chondriosomes and plastids. So all cytologists are at present agreed in attributing to the chondriosomes some important rôle in cellular metabolism.

The rôle of the plastids in chlorophyll-bearing plants is, on the contrary, very clear, for it is manifested morphologically by the production within these organelles of chlorophyll, carotinoid pigments, starch grains and so forth. However, we do not know at all by means of what physico-chemical processes these phenomena are brought about. At first an essential rôle in the elaboration of these different products, as well as participation in the phenomenon of photosynthesis, was attributed to the plastids. The plastids were considered as small laboratories which were the seat of the most important synthesis in the plant cell.

At the present time there is a tendency, rightly or wrongly, to react against this way of thinking and to consider the plastids as perhaps only the accumulation centers for certain substances manufactured by the cytoplasm itself. In any case, the work of modern physiologists caused it to be thought that the plastids contribute only in part to photosynthetic phenomena. Actually, we still do not know how much to attribute to the chlorophyll, how much to the substratum of the plastids and how much to the cytoplasm. Nevertheless, it seems that the plastids do indeed play an important part in photosynthesis. In any case, there is a rôle which cannot be denied the plastids. It is the ability to condense the hexoses into starch. In this case it is a question not of accumulation but of actual synthesis. The influence of plastids on the form and growth of starch grains is manifested by the fact that these properties are determined by the place where the grain appears in the plastid. If the grain arises in the middle of the plastid, the hilum, *i.e.*, its oldest part, is the center and concentric layers, which are the outcome of growth by apposition, develop regularly about it. The starch grain in this case remains entirely enveloped by a thin mitochondrial layer. When, on the contrary, the growing grain forms on the periphery of the plastid, it very soon bursts out of the plastid which then covers it as a sort of cap at only one extremity. In this case the hilum is situated at the pole opposite to that occupied by the cap, and the layers of growth do not form any longer, except in those regions which are still in immediate contact with the plastid. The grain then is eccentric in structure.

The fact that the starch grain is formed and is hydrolyzed in the interior of the plastid led certain investigators to believe in the existence in the substratum of the plastid of a diastase of reversible action, capable of bringing about both synthesis and hydrolysis of starch (MEYER, SALTER). MAIGE, on the contrary, thinks

that there is in the plastid only a synthesizing diastase and that the hydrolyzing diastase has its seat in the cytoplasm. The existence of diastase is, however, not necessary, if the hypotheses are accepted as formulated by NAGEOTTE and DEVAUX who consider the plastids and chondriosomes as catalysts.

It is known, moreover, that plastids in the embryo sac of *Lilium candidum* may enclose protein crystalloids which are used as reserve products. Some experiments seem to indicate that the plastids have the ability to accumulate proteins and it is even possible that they may be very important synthesizing centers (ULLRICH, GRANICK, etc.). Recently VOLKONSKY seems definitely to have furnished proof that the reticulate leucoplast of *Polytoma uvella* undergoes considerable variations in volume depending on the nature of the nutriment furnished it. It expands greatly in media rich in assimilable nitrogen. The leucoplast seems, therefore, to be the region of the cell to which nitrogenous nutrients most readily go, especially the amino acids, which are there transformed into more complex products. This phenomenon is to be considered in connection with the observations of NOËL on chondriosomes in livers of mammals, and seems to confirm the hypothesis of ROBERTSON-MARSTON, of which more will be said later. Yet VOLKONSKY says that this synthesis does not go beyond polypeptides and that the formation of proteins is completed in the vacuoles.

It is seen that, in reality, we are still very insufficiently informed on the rôle of plastids. The close relationship of the plastids and chondriosomes leads us to suppose that the two categories of elements must have a single function which is very general and that the function manifested morphologically by the plastids is only a special example of it. Thus, while admitting with the majority of cytologists that the chondriosomes have an important rôle in metabolism, we can, at the same time, examine the various hypotheses which have been proposed to explain the rôle of plastids and that of chondriosomes and which may apply to both categories of elements.

Purely for historical interest, the theory formulated in France by PORTIER (1919), then in America by WALLIN (1922) may first be mentioned. This theory held that the chondriosomes and plastids represent symbiotic bacteria which are found present in all cells and by means of which all syntheses take place in the cell. It was based solely on the morphological resemblance of the chondriosomes to bacteria and on the fact that with mitochondrial technique the symbiotic bacteria which are encountered in certain cells, notably those in the bacteria-containing root nodules of the legumes, stain like the chondriosomes. The theory is untenable, for even if it is true that the symbiotic bacteria stain as the chondriosomes do by these techniques, it signifies nothing since these stains are not specific and since symbiotic bacteria show histochemical behavior which makes it impossible to confuse them with chondriosomes (resistance to alcohol, acetic acid, etc.). This theory, successfully opposed by LAGUESSE, REGAUD, COWDRY and OLIT-

SKY, DUESBERG, LEVI, and MILOVIDOV, is today abandoned by PORTIER himself. Nevertheless it had the merit of initiating investigations which have produced methods by which chondriosomes can be distinguished in cells from symbiotic and parasitic bacteria. COWDRY and OLITSKY, DUESBERG, and MILOVIDOV have described methods by which, in the cells of nodules of legumes and in the adipose cells of cockroaches, symbiotic bacteria can be distinguished from the chondriosomes by means of differential staining. By these methods MILOVIDOV found that the symbiotic bacteria and the chondriosomes, including the plastids, are both distributed to the daughter cells during mitosis but not in the same manner. He has demonstrated, besides, that centrifuging brings about a displacement of the symbiotic bacteria in the direction of the centrifugal force but has no influence on the chondriosomes and plastids. The symbiotic bacteria, therefore, are heavier than the cytoplasm and are heavier than the chondriosomes and plastids. (Figs. 80, 81).

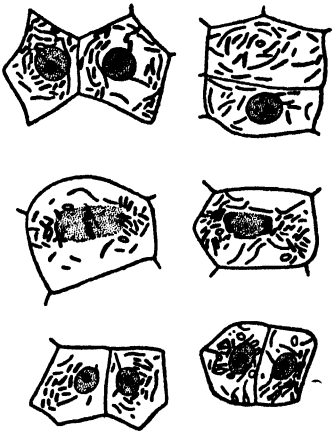


FIG. 80. — Cells of lupin. Bacteria and chondriosomes differentially stained. The bacteria (grey) cluster at the poles during mitosis, the chondriosomes (black) surround the chromatic spindle. (After MILOVIDOV).

This work on animal cells led REGAUD to consider the chondriosomes as "organelles having an eclectic and pharmaceutical function in the cell" *i.e.*, as "electosomes". According to this theory, the chondriosomes by means of a physico-chemical mechanism still unknown, draw from the surrounding medium the materials necessary to the life of the cell, transform them and finally release the product of elaboration, so that it may be excreted or kept in reserve. An analogous theory was applied by P. A. DANGEARD to his "vacuome" which he likened to the chondriome.

MAYER and SCHAEFFER, basing their idea on reports according to which the fatty acids contained in the lecithins are made to play the rôle of self-oxidizing bodies have suggested that the chondriosomes, by virtue of their lipoprotein constitution, might be the center for the very general function of reduction and oxidation and, in this way, have a rôle to play in the respiratory phenomena and indirectly in cellular synthesis.

Along these same lines may be mentioned reports of a certain number of authors who have considered that the chondriosomes and plastids are the source of various diastases or other substances playing an important rôle in the oxidation-reduction process in cells. Various workers have revealed in the chondriosomes the presence of oxidases or peroxidases (MARINESCO, PRENANT, MANGENOT). CHODAT and ROUGE found that in plant cells the oxidases are localized in the plastids. In these last years JOYET-LAVERGNE

and GIROUD have maintained that glutathione is found in the chondriosomes. JOYET-LAVERGNE reports having localized vitamin A in the chondriosomes and in the plastids, but he does not seem to have brought forward sufficient proofs for this localization. He even reports having proved by means of certain reagents that the oxidation-reduction capacity of the chondriosomes in the Saprolegniaceae depends on the presence of this substance in the substratum. As a matter of fact, the reactions used to detect the presence of glutathione in cells are not such as to permit it to be localized in the cytoplasm with any accuracy. At the present time there do not seem to be any microchemical reagents which can localize glutathione in the chondriosomes and the hypothesis of JOYET-LAVERGNE has not been verified. In fact, we shall see that the chondriosomes do not seem to have of themselves any reducing power. In any case, they are incapable of reducing Janus green to its leucoderivative, contrary to what has been thought up to now. As for the oxidizing rôle of these elements, it has not been confirmed either.

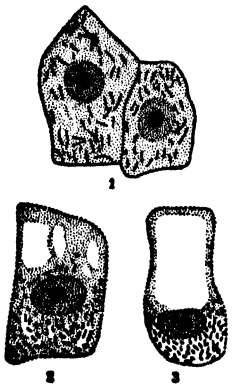


FIG. 81. — Fixed and stained cells of lupin nodules. 1, bacteria resembling chondriosomes. 2, 3, cell centrifuged before fixing and staining; bacteria, heavier than cytoplasm, left at one side. (After MILOVIDOV).

GIROUD and his collaborator report having localized ascorbic acid, also, within the chondriosomes of animal cells by using an acid solution of silver nitrate. These investigators moreover, attributed the Molisch reaction (pp. 54, 104) which characterizes the chloroplasts and which they obtained by the same reagent, to the presence of ascorbic acid in the chloroplasts which are thus reported to be the locus of this substance. To verify this hypothesis, GIROUD and his collaborators measured the quantities of ascorbic acid present in a great number of plants, and found an evident relation between the presence of chlorophyll and that of ascorbic acid.

This conception, accepted by various investigators, MIRIMANOFF was not able to confirm in his recent research. By measuring in the organs of a rather large number of plants, very accurate quantities of ascorbic acid, he was not able to obtain any direct relation in these plants between the content of ascorbic acid and that of chlorophyll. There are organs, totally deprived of chlorophyll, which are nevertheless very rich in ascorbic acid and, conversely, there are those poor in the acid which enclose a great deal of chlorophyll. If sometimes a relation is found between the presence of chlorophyll in the plastids and their richness in ascorbic acid, this is, consequently, only indirect and can be explained only by saying that ascorbic acid, like many other substances manufactured in plants, is an indirect product of photosynthesis. Moreover, MIRIMANOFF has shown that the reaction used by GIROUD is not specific for ascorbic acid in plants in which very frequently

there exist phenolic compounds (tannins, oxyflavanol and anthocyanin pigments) capable of reducing silver nitrate. Moreover this reagent is never reduced by the leucoplasts, the chromoplasts, or the chondriosomes in tissue which is, nevertheless, rich in ascorbic acid but lacking in chloroplasts and there is reason to believe that ascorbic acid is localized in the vacuoles. MIRIMANOFF thinks that the Molisch reaction has a totally different significance from that attributed to it by GIROUD. He thinks that it may be compared to a photolysis, the activator being chlorophyll, the hydrogen donator, glucose.

In addition, it has been supposed that the chondriosomes and plastids are chemical catalysts. From this point of view a first hypothesis was formulated by NAGEOTTE to explain both the rôle of the genuine chondriosomes and that of the plastids of green plants. It is based on research in plant cytology which has demonstrated that the plastids, regarded as a special category of chondriosomes, are not destroyed during their operations, and it attributes to chondriosomes and plastids the rôle of heterogeneous catalysts. The homogeneous catalysts according to this theory are represented in the cytoplasm by the diastases and the heterogeneous catalysts by the chondriosomes and plastids.

DEVAUX later formulated a different hypothesis, according to which the rôle of catalyst is played by the interfaces between the chondriome and the cytoplasm. DEVAUX formulated an interesting suggestion which gives significance to the heterogeneous structure of the cell and brings out its real importance. He demonstrated that all solid parts of the cell are orientated molecularly. Each molecule or elementary particle not only occupies there a definite, but an oriented, position. All the poles of like affinities occupy one face of the membrane while all the opposite poles occupy the other face. These facts made it possible for him to conclude that the plasmic membranes in particular must constitute the principal tools of the protoplasm. Applying these data to facts brought out by cytologists, DEVAUX, in order to explain the prodigious work which goes on in the cell, alleges surface actions which may be reduced to, or classified as, the operations of surfaces in the protoplasm. Now all protoplasmic surface, external or internal, is characterised by the formation of a coagulation membrane. The polarized (catalytic) membranes are the scene of cellular activities (activation by surface agency). There is a catalytic localization of protoplasmic activity on the surfaces presented by the protoplasm: between the cytoplasm on the one hand, and the nucleus, chondriosomes, plastids, vacuoles and cellular membrane on the other.

This hypothesis is based on the fact recognized by OTTO WARBURG, that, in the eggs of the sea urchin, respiration takes place essentially all along the protoplasmic membrane. WARBURG applied this notion to the chloroplasts, whose activity he considers to be purely a surface activity. DEVAUX says, "It must be concluded that the cell is a system of numerous catalysts in the form

of small closed sacs, automatically forming and maintaining themselves at the same time that they produce all the physico-chemical transformations taking place in the cell. This establishes the bond, heretofore mysterious, between cellular structure and vital activity."

COWDRY and LECOMTE DU NOÜY have shown, moreover, by meticulous measurements, that the surface of the chondriosomes is greater than that of the nucleus, although the total nuclear volume is about five times greater than the total chondriosomal volume of the same cell. The mitochondrial substance seems therefore to realize a maximum surface with the minimum of material and these investigators think that on these interfaces of considerable area, certain substances may accumulate and the concentrations attained may allow pluri-molecular reactions of the very highest importance to take place between the interfaces.

ROBERTSON has formulated a theory similar to that of DEVAUX based on the data brought out by MARSTON who showed that dyes of the azine series, such as Janus green, have a precipitating action specific for proteases and that, moreover, the action of these enzymes is doubled in the presence of an emulsion of lecithin, the surface of the lecithin serving as the catalytic surface. ROBERTSON thinks, therefore, that the staining of the chondriosomes by Janus green is an index of the presence of proteases in their substratum and that the chondriosomes enclose proteases of reversible action, capable of bringing about both the synthesis and the hydrolysis of proteins. He believes that the chondriosomes may be the site of proteosynthesis, a synthesis which takes place by virtue of the lipide surface of the chondriosome which acts as the catalytic surface. In the course of his research on the vacuolar system of animal cells, PARAT noticed that the cytoplasm and the chondriosomes seem to have a reducing power, while the vacuoles seem to have an oxidizing power. This investigator is thus led to formulate the hypothesis that the chondriome brings about oxidation-reductions by means of which cellular synthesis is carried out, and that the second phase, or respiratory oxidation, which follows these phenomena occurs in the vacuoles. Having observed, moreover, that the chondriosomes and the vacuoles are often in intimate contact, PARAT supposes that the combination, chondriome + vacuome, is responsible for protein synthesis which, according to ROBERTSON, entails a lipide phase and an aqueous phase. This author thinks, also, that the vacuolar system may be the region in which operations begun in the chondriome are completed. Recently Miss LE BRETON, basing her ideas on those of ROBERTSON and MARSTON, and on investigations of JOYET-LAVERGNE, GIROUD, and PARAT, sought to show that chondriosomes are found in the conditions necessary and sufficient for them to be the center of protein synthesis.

Recent work (GUILLIERMOND and GAUTHERET) does not confirm PARAT's hypothesis. The fact that chondriosomes stained vitally with Janus green lose their color rather quickly, a fact

observed by many authors (GUILLIERMOND, PARAT, SOROKIN) had led to the acceptance of the idea that chondriosomes have the power to reduce Janus green to its leucoderivative. It will be seen that this interpretation is inexact and that the chondriosomes are incapable of carrying out this reduction. All that they do is to reduce Janus green to its rose derivative, which, having less affinity for the chondriosomes than for the cytoplasm or the vacuole, diffuses into these latter. The chondriosomes, moreover, share this property with the vacuole when the latter contains substances capable of holding the dye and often the reduction even begins in the vacuoles and is completed in the chondriosomes. On the whole, Janus green is reduced wherever it is localized and the chondriosomes do not seem to have a more active rôle in this reduction than the other elements of the cell. The chondriosomes can not then be considered as having a reducing capacity and the vacuoles as having an oxidizing capacity.

Nothing is positively known about the rôle of the chondriosomes. One fact, however, stands out from recent research. This is that the chondriosomes, like the leucoplasts, have the property of being stained selectively and in a transitory manner by a rather large number of vital dyes, which dyes later accumulate in the vacuole. It could therefore be supposed that they behave in the same way in the absorption of various substances which the cell takes from the external medium. According to this theory, these substances are taken up by the chondriosomes, then thrown off into the vacuole, either directly, or after having undergone some transformation (synthesis), this transformation being brought about during contact with the chondriosomes, by the mechanism suggested by DEVAUX. This hypothesis, which seems to agree both with that of REGAUD and that of DEVAUX, explains the accumulation of protein by the chondriosomes of the liver when an animal is subjected to an exclusively nitrogenous diet (R. NOËL) and explains the capacity of leucoplasts to take up amino acids (VOLKONSKY).

Apart from these hypotheses, it has been thought, also, that the chondriosomes and the plastids might have a rôle in the phenomena of heredity. This was the opinion of MEVES. From work carried out on plants with variegated leaves and branches, that is to say plants presenting a mosaic of green leaves and colorless parts lacking chlorophyll, various authors suggested a rôle that the plastids seem to have in heredity. Thus, to cite only one example, a variegated hybrid of *Oenothera* (*O. rubridivaricata*), can form flowers at the extremity of green branches and at the ends of colorless branches. Now, after pollinating the flowers on the uncolored branches by the pollen of flowers on uncolored branches, RENNER obtained entirely colorless seedlings. The pollination of flowers of uncolored branches by pollen from flowers on green branches has given a mixture of colorless seedlings, variegated seedlings and normal green seedlings. In a second series of experiments, RENNER pollinated flowers from green branches by

pollen of flowers produced on colorless branches. Most of the descendants of such a cross are green, some are variegated but none are colorless. To explain these data, the hypothesis has been put forth that the oospheres and pollen grains within flowers growing on uncolored branches contain only plastids incapable of becoming green. In the first series of experiments, the oospheres with modified plastids reached by pollen tubes with plastids equally modified, give rise to colorless plants; when reached by pollen tubes containing normal plastids, they produce variegated or green plants because a small number of plastids of male origin have penetrated the oosphere. During the succeeding divisions the normal plastids are distributed by chance. When some cells receive only these normal plastids, while others receive modified plastids of maternal origin, variegated plants are obtained. When the normal plastids, although brought in in very small numbers, are distributed among all cells of the embryo, an entirely green plant is the result. Finally, when normal plastids, having been brought in in very small numbers remain in a negligible quantity, colorless plants are obtained. In the second series of experiments, colorless plants were never procured because in this case the cytoplasm of the oosphere, which is always in excess of that delivered by the pollen tube, contains normal plastids. The few variegated seedlings give evidence of modified plastids having been brought in by the pollen tube. Finally, to account fully for the differences between the results obtained in the two series of experiments, it must be admitted that the normal plastids, capable of becoming green, increase in numbers more rapidly than do the modified plastids. That is why the latter, although brought in in large quantity by the pollen tube, are never represented exclusively in one plant.

The hypothesis is evidently plausible but it has no cytological basis—at least up to the present—for we have seen that the behavior of the plastids and chondriosomes in fertilization is not known.

Chapter XII

THE VACUOLES

Early data. Insufficiency of methods. Theory of Hugo de Vries:- Plant cells always contain, in their cytoplasm, watery inclusions to which have been given the name *vacuoles* and which are the regions of accumulation of numerous metabolic products. The vacuoles contain a liquid called the *vacuolar sap* which holds in solution very diverse crystalloid substances, such as mineral or organic salts, mineral acids, sugars, and so forth.

In cells in the process of differentiation, several vacuoles are generally found but in most mature cells there is only one enormous vacuole which occupies the greater part of the cell, the cytoplasm forming about it only a thin parietal layer containing the nucleus appressed to the cell wall.

The splendid investigations of the Dutch botanist, HUGO DE VRIES, brought out the essential rôle of vacuoles in osmotic phenomena of the cell and showed that a mature plant cell may be likened to a small osmometer, composed of a semi-permeable ectoplasmic layer which lines the permeable cell wall on the inside, and of a vacuole containing a solution of crystalloid substances. No great modification is observed to take place if mature plant cells are placed in water, for example, a staminate hair of *Tradescantia* which has the advantage of being easily detached and of being made up of large cells which lend themselves easily to observation. However, it can often be observed that the vacuole dilates and by its pressure causes a curvature of the cell wall. The vacuolar sap, by virtue of the crystalloid substances which it holds in solution, has an osmotic pressure superior to that of pure water. It is, therefore, hypertonic to water and so endosmosis takes place. If the same cells are plunged into a solution hypertonic to the vacuolar sap, these cells then show a remarkable phenomenon discovered by DE VRIES and called by him *plasmolysis*. There is produced an exosmosis which causes a contraction of the protoplasm. The protoplasm becomes more and more detached from the cell wall, and soon forms in the center of the cellular cavity a globular mass separated from the cell wall, but remaining connected with it by very fine cytoplasmic trabeculae which give evidence of adherence of the cytoplasm to this wall. In elongated cells the protoplasm as it contracts divides into several globular masses connected like beads on a string by a thin cytoplasmic filament. This phenomenon is caused primarily by the exit of water from the vacuole, accompanied, of course, by a lack of imbibition on the part of the protoplasm, but it is the contraction of the vacuole which brings about the contraction of the protoplasm. Whenever the cells on which the experiment is being carried out contain in

their vacuoles an anthocyanin pigment which gives them a natural color, it is observed that as the cells become plasmolyzed the color of the vacuole is considerably accentuated. The pigment therefore is becoming concentrated in the vacuole.

The phenomenon of plasmolysis takes place only in living cells. A dead cell has become permeable and can no longer be plasmolyzed. Therefore the capacity of the cell for being plasmolyzed serves as a criterion as to whether the cell is living.

When carried out with certain precautions, plasmolysis does not cause the death of the cell and may be followed by the converse phenomenon, if the cell be placed in a solution of pure water. Endosmosis then occurs which again brings about the dilation of the vacuole and the cytoplasm once more becomes pressed to the cell wall. The cell now recovers its normal aspect. It is said to have been *deplasmolyzed.*

As the ectoplasmic layer which lines the interior of the cell wall is generally only relatively semi-permeable, plasmolysis is followed, at the end of a period varying according to the case, by a spontaneous deplasmolyzing action which is brought about by the gradual penetration into the vacuole of the substance dissolved in the surrounding medium. Thus there is re-established an osmotic equilibrium between the vacuolar sap and the external medium.

If plasmolysis is brought about too brusquely, however, it leads to the death of the cell. As soon as this happens, the ectoplasmic layer disorganizes and water enters the cellular cavity. The protoplasm is coagulated and the cell cavity now contains nothing but protoplasmic coagulations floating in the water which has accumulated within the cell cavity. Now, during this phenomenon the vacuole, which is more resistant, remains stretched out in its habitual shape and, if it contains anthocyanin, this pigment remains localized within the vacuolar sap. The vacuole appears to be separated from the medium by a limiting semi-permeable layer. This layer is resistant much longer than the ectoplasmic layer. The vacuole may thus be preserved for a very long time. Then its limiting layer is destroyed in turn, and the contents of the vacuole blend with that of the cell cavity. In this way the vacuole may subsist within the cell cavity after the death of the cell. This phenomenon DE VRIES called the *isolation of the vacuole,* a classical phenomenon which has been seen since by numerous investigators: TSWETT, GUILLIERMOND, KÜSTER, WEBER, HÖFLER, EICHBERGER, etc.

This phenomenon demonstrates that the vacuole is limited externally by a peripheral, semi-permeable layer. It is of the same nature as that which surrounds the outside of the cytoplasm and is called the *endoplasmic layer,* or *perivacuolar layer.* It is however much more resistant than the outer cytoplasmic layer.

As a result of his work, DE VRIES was led to consider this semi-permeable layer as a differentiated membrane and the vacuoles as permanent components of the cell. He considered that the vacuoles are made up of vacuolar sap, containing in solution various crystalloid substances, are surrounded by a semi-permeable, differen-

tiated membrane, and are endowed with properties of secretion. It is this membrane to which DE VRIES gave the name *tonoplast*, which he believed secreted all the substances dissolved in the vacuolar sap. DE VRIES, moreover, thought that the vacuoles can not in any case form *de novo*, but are always transmitted by division from cell to cell, like the nucleus and plastids. In short, DE VRIES compared the vacuoles to a sort of liquid plastid to which VAN TIEGHEM gave the name *hydroleucites*.

At the time that DE VRIES did his work, however, the origin of vacuoles was unknown. All that was known, and had been known for some time, was that several vacuoles appear scattered about in the cytoplasm of cells which are beginning to differentiate; that they enlarge and then coalesce until in mature cells there is only one enormous vacuole occupying the entire cell, pushing the cytoplasm and the nucleus back to the periphery. Aside from this, nothing was known. As a matter of fact, it is generally impossible to distinguish the vacuoles in living embryonic cells. Hence, in the opinion of the earlier botanists, the vacuoles were lacking in these cells and formed *de novo* in the course of cellular differentiation, as seems to be indicated in figure 82, reproduced here from SACHS.

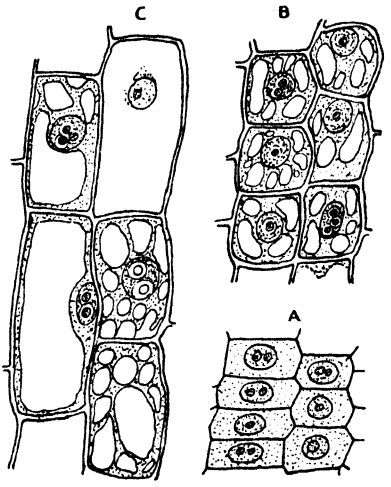


FIG. 82. — *Fritillaria imperialis*. Development of vacuoles in the cortical parenchyma. (After SACHS).

WENT, a student of DE VRIES, succeeded, however, in revealing in embryonic cells of certain plants, the existence of small vacuoles which increase in numbers by fission. This seemed to indicate that the vacuoles do not arise *de novo*, but keep their individuality during the course of development, and consequently seemed to support the thesis of DE VRIES.

This theory, nevertheless, was not based on sufficiently solid facts. It has not, as a matter of fact, been possible to bring out the vacuolar membrane by means of stains and its existence is manifested only by its property of semi-permeability. It is true that there is the phenomenon of isolation of the vacuoles, which obliges us to admit that there exists, at least in mature cells, a semi-permeable membrane about the vacuoles, more resistant than the rest of the cell. CHAMBERS and HÖFLER who studied this membrane during micromanipulation, describe it as a membrane of inappreciable thickness, very cohesive and extensible, formed of a substance non-miscible with water. The significance of the perivacuolar layer is still very much disputed. Some observers regard it as a differentiated membrane (HÖFLER), others as formed by a

coagulation of the cytoplasm which is in contact with the vacuoles. PEKAREK thinks it is protein in nature. WEBER thinks it does not belong to the cytoplasm but to the vacuole and is the outcome of a condensation about the vacuole of the lipides found in solution within it. According to EICHBERGER, this perivacuolar layer is of the same nature as the ectoplasmic layer, composed, like it, of proteins and lipides but much more resistant. He examined the layer over a period of several days in abiotic solutions, for instance in a 5% solution of copper sulphate and over a period of from two to three hours in a 20% solution of calcium citrate, which proves that it acts like an inert membrane and not like a membrane differentiated and living, in the sense of DE VRIES.

On the other hand, it is very difficult, if not impossible, to observe the vacuoles in embryonic cells and consequently to demonstrate that they do not form *de novo*. Therefore the theory of DE VRIES and WENT was contested by numerous botanists, among others, PFEFFER and NĚMEC. PFEFFER, in particular, showed that it is possible to produce artificial vacuoles in the plasmodium of *Chondrioderma difforme* by placing it in a solution saturated with asparagin. The plasmodium encircles the crystals of asparagin, which, dissolving in the cytoplasm, form there vacuoles which are indistinguishable in all ways from the pre-existing vacuoles. For this reason, PFEFFER thinks that every particle found in the cytoplasm which will take up water more readily than it, is capable of producing a vacuole. It is true that PFEFFER's experiment does not demonstrate very much, for here it is a question of the production of digestive vacuoles which are perhaps not the same as the other vacuoles.

The question of the origin of the vacuoles remained uncertain for a very long time, because neither observation of living material nor fixed and stained preparations made it possible, in general, to follow the development of these elements. Vacuoles in living embryonic cells can not ordinarily be distinguished and in fixed preparations the vacuoles are always altered by swelling.

Rapid progress in this field was not made until very recently when investigators began to use vital stains.

Chapter XIII

VITAL STAINING OF THE VACUOLES

Colloidal substances in the vacuolar sap:- The existence of dyes, called vital dyes (methylene blue, cresyl blue, Nile blue, neutral red, etc.) has been known for some time. They have the property of penetrating living cells and of coloring some of the cytoplasmic inclusions.

PFEFFER first showed that methylene blue used in a 1% solution penetrates the cells of various plants (*Azolla, Lemna, Spirogyra*) but accumulates exclusively in the vacuole and does not color the protoplasm. Methylene blue brings about dark blue precipitates in the vacuoles as a result of the flocculation of the tannins contained in the vacuolar sap, with which the stain forms a complex. PFEFFER stressed the remarkable property which the vacuoles possess, of accumulating methylene blue and of taking on very rapidly a coloration more intense than that of the solution of the dye itself. The dye becomes, therefore, more concentrated in the vacuole than it was in the solution. In staining tadpoles, PFEFFER showed that methylene blue accumulates in certain inclusions of the cytoplasm. Since that time it has been demonstrated that other dyes, such as cresyl blue, Nile blue and neutral red, also have the property of penetrating living cells, and numerous investigations on plant cells as well as on animal cells have determined that these dyes accumulate exclusively in the inclusions which are not a part of the living matter (secretion granules of animal cells, vacuoles of plant cells).

In research on yeasts, we ourselves have demonstrated that the vacuoles enclose granules, showing Brownian movement, which have the property of being stained instantaneously by cresyl blue and neutral red. These granules can be easily fixed with alcohol and formalin, after which they are stained deeply by basic stains (anilin blue, anilin violet and haematein) which give them a reddish tint. This has permitted us to identify them as *metachromatic corpuscles*. These were cited first in the bacteria by BABÈS and found later in the Cyanophyceae by BÜTSCHLI, who gave them the name of "red granules". In later research also, we showed that vacuoles formed by hydration of aleurone grains (Fig. 84), when seeds germinate, accumulate neutral red and this dye brings about the flocculation of the proteins which the vacuoles contain in solution. But these are isolated observations. It remained for P. A. DANGEARD to establish the fact that the ability to accumulate vital dyes is a general property of vacuoles.

Taking up our work on the metachromatic corpuscles in the fungi and algae, DANGEARD demonstrated that these bodies are visible only rarely in living cells and when they are visible, they

always appear in much smaller numbers than when obtained by vital staining or after fixation. These bodies, therefore, are generally the result of flocculation of a substance found normally in a colloidal solution in the vacuolar sap. This flocculation occurs in the presence of vital dyes or fixatives. P. A. DANGEARD kept for this substance the name *metachromatin* which we had proposed to designate the substance constituting the metachromatic corpuscles (*volutin* of ARTHUR MEYER).

P. A. DANGEARD had the idea of trying the effect of vital stains, among others cresyl blue, on a very large number of cells of the most varied plant groups. In every one he found that there existed a colloidal substance dispersed in the vacuolar sap possessing a strong capacity for taking up vital stains which precipitate it in the form of corpuscles showing Brownian movement. These he identified with the metachromatin of fungi. Thus he arrived at the conclusion that all vacuoles enclose metachromatin, a specific substance of vacuoles, and he states that vital staining thus constitutes a property of the vacuoles which is both general and characteristic.

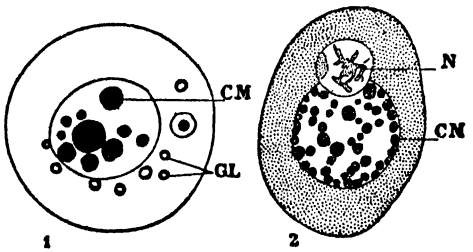


FIG. 83. — *Saccharomyces cerevisiae*. Precipitation of metachromatin corpuscles (*CM*) in vacuole; 1, by neutral red; small vacuole at right contains one corpuscle. 2, haematein, after fixation with formol; *GL*, very refractive lipide granules. *N*, nucleus.

Studying the origin of vacuoles in most varied plant cells by staining them with the vital dye, cresyl blue, DANGEARD demonstrated that vacuoles exist in all embryonic cells but are very different in appearance from ordinary vacuoles. They are seen here as numerous minute elements composed of a very concentrated solution of metachromatin in a semi-fluid state. By their forms, as well as by their dimensions, they are decidedly reminiscent of the chondriosomes. It is these elements, swelling by imbibition and coalescing, which finally become the large vacuoles characteristic of mature cells. We shall not dwell on this matter here, but will study it later in more detail.

Our research immediately afterward, confirmed, in part, the facts observed by P. A. DANGEARD. We recognized that the metachromatin of fungi is normally found in a colloidal solution in vacuoles. Vital dyes, for example neutral red, precipitate this substance as corpuscles, intensely stained and showing Brownian movement in the vacuolar sap which either remains colorless or takes a diffuse stain. We also found, by using vital dyes in the most diverse plant cells, that colloids are present as precipitable pseudosolutions. Contrary to DANGEARD's opinion, our work showed, as we shall see further on, that the colloidal substances contained in the vacuoles are of very different chemical natures and do not show in the higher plants, nor even in many of the lower

plants, the characteristic properties of metachromatin, with which they have in common only the ability of forming absorption complexes with vital dyes.

It is well established, therefore, that the affinity of the vacuoles for vital dyes, with some exceptions which will be taken up later, is a general property of vacuoles and that this is due, not to the presence in the vacuoles of a specific substance corresponding to metachromatin, but to colloidal substances whose nature may vary radically, depending on the species in question.

The presence of colloids in the vacuoles has, moreover, been confirmed by another method. This has been employed by WEBER, FREY, and REILHES, who tried to determine the degree of viscosity of the vacuolar sap by observing the rapidity with which certain solid bodies (calcium oxalate or calcium sulphate crystals, lipide concretions) contained in the vacuoles fall in their liquid when the microscope is inclined. FREY was able to show by this method that the viscosity of vacuolar sap of *Closterium* cells at 18° C. is about twice as great as that of water. This viscosity increases considerably in dead cells. After cells have been fixed, it becomes impossible to obtain a fall of the calcium oxalate crystals contained within their vacuoles. This can be explained only by the fact that they are surrounded within the vacuoles by coagulated colloids which prevent them from moving.

Finally, the more recent research of WEBER on "vacuolar contraction," which will be taken up later, brought forward new data in favor of the presence in the vacuoles of colloidal substances which may be true gels.

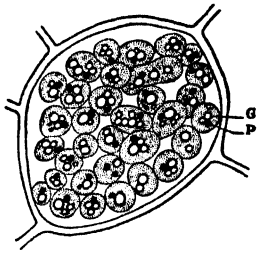


FIG. 84. — *Zea Mays.* Living cell stained with neutral red, from the aleurone layer at the beginning of germination of the seed; aleurone grains have become diffusely staining vacuoles containing one or more deeply stained protein bodies (*P*) and one or more colorless globoids (*G*).

Action of vital dyes on the cells. Advantages of vital staining:- When colloidal substances endowed with an affinity for vital dyes had been demonstrated to be generally present in vacuoles, there remained the task of determining more specifically the action of these dyes on the cells. Do they accumulate exclusively in the vacuoles or may they stain at the same time other cytoplasmic inclusions? Have they an injurious action on the cells and are they not capable of altering the shape of the vacuoles? Or may they not even bring about the appearance of artificial vacuoles in the cytoplasm? These are questions which were raised. It was all the more important to elucidate them since the vacuoles of embryonic cells appear as minute elements with very concentrated colloidal contents which usually can be revealed only by the use of vital stains. Very accurate work carried out recently on vital staining—our own work carried out over a period of twenty years and especially our recent work in collaboration with GAUTHERET—

has shed considerable light on this question. The essential results will be summarized here as briefly as possible.

It appears, as KÜSTER had already noticed, that living plant cells are permeable to a great number of vital dyes. These may accumulate in all the vacuoles, whatever their content. Others, such as methylene blue, Bismarck brown and chrysoidine, may stain the cytoplasm and the nucleus and may accumulate in the vacuoles, but only if the vacuoles contain phenol compounds (tannin, oxyflavanol and anthocyanin pigments). The cytoplasm and nucleus are particularly easy to stain with chrysoidine.

In general it is only the basic dyes which penetrate living cells. Under some conditions, however, some acid dyes will do this, but the greater number of the dyes are not of importance in the question which occupies us.

There are only a small number of vital dyes, all of them basic, which are capable of being used for the study of morphological constituents of the cytoplasm and these may be divided into two groups from the point of view of their action on plant cells:

1. Dyes, which like Janus green, Dahlia violet, methyl violet and a certain number of others, at first stain the chondriosomes and plastids, for which they have an affinity, but can also under some conditions accumulate in the vacuoles. (Among these dyes, Bismarck brown and methylene blue are very slightly toxic. We have been able to germinate grains of wheat and have made *Saprolegnia* grow in media to which these dyes have been added in proportions from 0.0005-0.02%. In young wheat roots, as well as in *Saprolegnia,* the vacuoles which stain only between slide and cover glass and only under certain conditions (especially when they enclose phenolic compounds), accumulate the dyes during growth. Chrysoidine, beginning with solutions of 0.005% is, on the contrary very toxic).

2. Dyes, which, like neutral red, neutral violet, cresyl blue, Nile blue, naphthylene blue and naphthylamine blue, do not stain the chondriosomes and plastids but, under normal conditions, accumulate exclusively in the vacuoles.

The dyes of the first group are very toxic and do not produce vital staining except when employed in solutions of low concentration. At higher concentrations they are taken up by the chondriosomes in cells which remain alive for some time as shown by their cytoplasmic currents, but in these cells the dyes rapidly cause death. The staining is, therefore, *sublethal.* Recent work (GUILLIERMOND and GAUTHERET) has shed a good deal of light on the question of vital staining of leucoplasts and chondriosomes, which until now had been rather obscure. This work has shown, as has been already seen, that a certain number of dyes may be taken up at first by the cytoplasm and nucleus, or by the chondriosomes and leucoplasts, but this staining is purely transitory and the dye goes into the vacuole very quickly. It is only when the cells have excreted the stain into the vacuoles that the cells are capable of growing and multiplying.

This work has demonstrated that the toxicity of the vital dyes which stain the chondriosomes is approximately the same for phanerogams as for fungi. The least toxic is Janus green. Germination of wheat seeds may be obtained in media to which 0.0005-0.001% of Janus green has been added. The roots grow well in solutions as high as 0.005% but at higher concentrations their growth is inhibited and they soon die. The other dyes are much more toxic. Wheat seeds will germinate in them, with the exception of methyl green and Victoria blue, only in solutions between 0.0002-0.0008%. The dye is never taken up by the chondriosomes and leucoplasts but accumulates only in the vacuole (Janus green generally in its rose form). *Elodea canadensis* can be kept alive in a 0.0005% solution of Janus green or of methyl violet. At the beginning, the dye is taken up only by the chondriosomes but after a short while these elements lose their color and the dye accumulates only in the vacuole (in its rose form in the case of Janus green).

It has also been possible in this work to follow the reduction of Janus green to its rose derivative as it occurs in the course of vital staining. If a strip of epidermis from a bulb scale of *Allium Cepa* is colored between slide and cover glass in a 0.0005-0.005% solution of Janus green, the dye is too dilute to produce a macroscopically visible staining of the epidermis, but a microscopic examination of the preparation shows that Janus green has been taken up by the chondriosomes and leucoplasts. After several minutes, these elements lose their color and there is no longer any trace of the dye to be seen in the cell. If the same experiment is repeated in solutions of 0.01-0.02% of Janus green, the strip of epidermis is stained green macroscopically but at the end of one or two hours, it changes to rose. Janus green has therefore been reduced to its rose derivative. This reduction is irreversible and the rose derivative can no longer by re-oxidation resume its green form. If air is excluded by sealing the slide with paraffin, reduction is more rapid and can be followed under the microscope in a single cell. At the beginning, the dye stains only the chondriosomes and leucoplasts, the chondriosomes more intensely than the leucoplasts, and accumulates at the same time in those vacuoles which contain oxyflavanol compounds. At the end of about half an hour in cells in which the vacuole is not stained, the chondriosomes and leucoplasts lose their green color at the same time that the cytoplasm and nucleus take on a rose tint. In cells in which Janus green has accumulated in the vacuole, reduction often begins there. The vacuole changes from violet to rose, more accentuated than the green stain that it first showed, while the cytoplasm and nucleus generally remain colorless. In reality the chondriosomes and leucoplasts only partially destain, for these two categories of elements show a pale rose tint. The cells which have reduced Janus green to its rose derivative can remain alive without air and show cytoplasmic currents for 48 hours. With concentrations above 0.02% of Janus green, reduction of the dye to its rose derivative is some-

times obtained but usually the cells die without having brought this about.

The study of the behavior of the rose derivative of Janus green in these cells explains these phenomena since the derivative does not behave like Janus green. Whereas Janus green stains only the leucoplasts and chondriosomes, or the vacuole if it contains oxyflavanol compounds, the rose derivative shows a greater affinity for the nucleus and cytoplasm, as well as for the vacuole containing oxyflavanol compounds, than it shows for the chondriosomes and leucoplasts which it stains only faintly. Therefore, if one uses a dilute solution of Janus green (0.0005-0.005%) the dye stains only

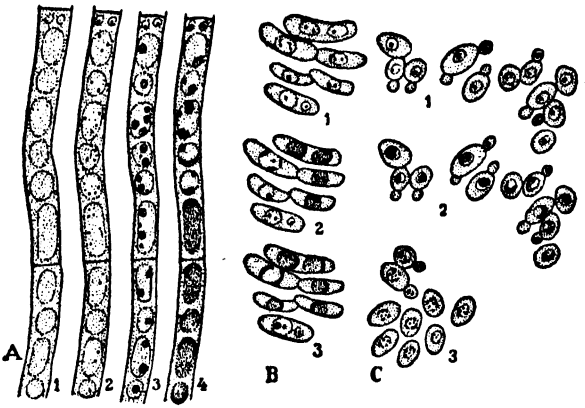


FIG. 85. — Vital staining with neutral red, except C3, observed under the microscope. A, *Penicillium glaucum*. 1, before staining; 2, small deeply stained precipitates in the vacuole showing Brownian movement; 3, fusion of small precipitates to larger bodies; 4, precipitates appressed to peripheral wall of vacuole, diffuse staining of sap. B, *Zygosaccharomyces Chevalieri*. 1, small precipitates in vacuole; 2, 3, fusion, bodies now appressed to wall of the vacuole, sap diffusely stained. C, *Saccharomyces ellipsoideus;* 1, 2, as in A, 1-2; 3, cells fixed by formol stained with cresyl blue which causes flocculation of the metachromatin from the colloidal substances in the vacuole as numerous, deeply stained bodies.

the chondriosome and leucoplasts but it stains them faintly. It is almost immediately reduced to its rose derivative, however, and this diffuses into the cytoplasm, the nucleus, or the vacuole, and its concentration is too weak to produce in them any appreciable coloration. On the contrary, a 0.01% solution of the dye taken up by the chondriosomes and leucoplasts, is reduced to its rose derivative which, being less strongly retained by these elements, diffuses into the cytoplasm and nucleus which it colors a pale rose in cells whose vacuoles are lacking in oxyflavanol compounds. In the case of the vacuole which, on the contrary, contains oxyflavanol compounds and has accumulated Janus green, reduction goes on at the same time in the chondriosomes and in the vacuole, and a part of the rose derivative formed in the chondriosomes diffuses into the vacuole. Often indeed, the reduction begins in the vacuole and takes place later in the leucoplasts.

This reduction is observed between slide and cover glass and in tissue incapable of development. It is probable that under normal conditions, *i.e.*, in culture or in an organ capable of growth, the rose derivative, once formed, accumulates in the vacuoles before the growth of the tissue.

It has been seen that in *Saprolegnia* similar phenomena take place. In many fungi, however, especially in the yeasts, Janus green may be taken up by the cytoplasm at the same time as by the chondriosomes, then be reduced there to its rose derivative (or even to its leucoderivative in anaerobic conditions) and this derivative is later excreted into the vacuole.

Among the dyes of the second group are some which are toxic, for example naphthylene blue and naphthylamine blue. There are others which are less so, such as cresyl blue and especially Nile blue. Finally, there are still others which are a very little toxic — neutral red and neutral violet, by far the least toxic of all these vital dyes. All these vital dyes produce coloration of the vacuoles which, as will be seen further on, is *essentially a vital phenomenon,* for it is possible only during the life of the plant.

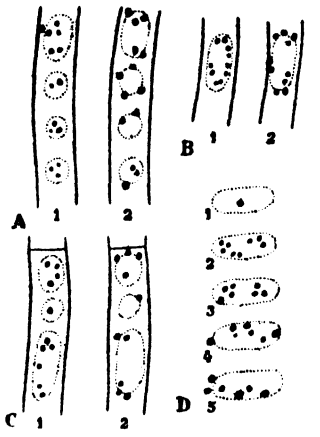


FIG. 86. — *Penicillium glaucum.* Vital staining with neutral red. A, B, C. 1, precipitates in vacuole; 2, migration of precipitates into cytoplasm. D, 1-5, stages observed in a single vacuole.

Neutral red and neutral violet are closely allied dyes. They behave in the same manner and are particularly interesting. From our research on the behavior of neutral red, it is found that except in rare cases, the dye is not retained by the cytoplasm and that the coloration of the living cell which results, is almost always strictly limited to the vacuoles (Fig. 85). We have found only a few rare yeasts in which neutral red may color certain lipide inclusions of a very special nature at the same time as the vacuoles, although we have examined a considerable number of cells belonging to the phanerogams and to the most diverse fungi observed with the aid of this stain. In the Myxomycetes, however, MANGENOT showed that neutral red colors certain sphaerocrystals of a phenolic nature. In the algae, some mucilaginous inclusions are known with which tannins are often associated, which are stained by the vital stains at the same time as the vacuoles. But it appears that these inclusions, to be taken up later, may be considered as vacuoles of a special nature.

The different phases of staining the living cell with neutral red may be followed under the microscope. The vacuoles, as we have said, appear in various forms. They may be small and contain colloidal substances in very concentrated solutions, as in embryonic cells, or they may be very large and contain a very dilute colloidal

solution as in differentiated cells. Neutral red behaves differently with the two types of vacuoles.

In the small vacuoles of very concentrated colloidal contents, neutral red does not cause any precipitation and stains deeply and homogeneously. These small vacuoles are not usually visible without the assistance of vital dyes. There are cases, however, in which they appear very distinctly because of the anthocyanin (red or violet) which they always contain and which gives them a natural color. Such is the case in the vacuoles of teeth of young rose leaflets (Fig. 92), which will be mentioned later. The study of these vacuoles in the living state makes it possible to see that they have exactly the same shape as those which are obtained by vital stains in cases where the vacuoles are not otherwise visible. There are cases in which the small vacuoles of meristematic cells, because of the high refractivity of their contents, are perfectly visible without vital dyes (barley root, wheat root, first leaves of the bud in *Iris germanica*). The staining of these small vacuoles with neutral red may be followed under the microscope and it may be observed that this staining is not accompanied by any alteration — on condition, of course, that the observation is not too greatly prolonged, for at the end of a certain time a swelling of the vacuoles always occurs.

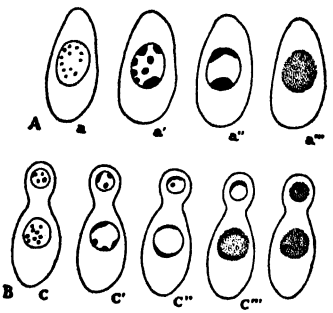


FIG. 87. — *Saccharomycodes Ludwigii*. Vital staining. Neutral red produces small precipitates in the vacuole, showing Brownian movement (*a*) which then fuse to form larger bodies (*a'*) sticking to the periphery of the vacuole (*a''*) and then dissolve, leaving the vacuole diffusely stained (*a'''*). B, similar series.

The large vacuoles with very disperse colloidal contents are, on the contrary, always visible without staining and it is very easy with the microscope to follow the different phases of their staining with vital dyes. The phenomena are very clear cut, particularly in the fungi (molds and yeasts). For example, by placing cells of *Saccharomycodes Ludwigii* grown in a van Tieghem and Le Monnier moist chamber (Fig. 88) in a drop of neutral red solution, it is observed that there are immediately produced in the vacuoles, a great number of granules strongly stained and showing Brownian movement. These are the result of precipitation of the vacuolar colloid through the action of neutral red. It sometimes happens that these precipitates, carried against the wall of the vacuole, pass through it and are deposited in the perivacuolar cytoplasm (Fig. 86), a phenomenon which is also caused by fixatives and which leads to errors of interpretation. This precipitation occurs even if an extremely dilute solution of neutral red is used. The reaction is, therefore, very delicate and the vacuolar colloid is very readily stained by the dye, but if the solution is very dilute, the phenomenon stops with the production in the vacuoles of small colored granules, showing Brownian movement. If, on the contrary, the solution is more concentrated,

the granules quickly coalesce into a small number of large globules (sometimes into a single globule) which come to be closely appressed to the wall of the vacuole. Then they diminish little by little in volume and disappear, while the entire vacuole takes on a diffuse stain which later becomes more pronounced.

To summarize these phenomena briefly: there is a precipitation of the vacuolar colloid, followed, when the stain is sufficiently concentrated, by a dissolution of the precipitate and by the homogeneous staining of the vacuolar sap.

These phenomena may be compared with those described by VON MÖLLENDORFF in animal cells. He found, in staining with neutral red the cells of the pronephros of amphibian tadpoles, that basic dyes stain the fluid and acid inclusions of the cytoplasm (corresponding apparently to small vacuoles) and cause the precipitation of the colloid of which they are composed, in the form of small precipitates. Then, if an excess of the electropositive dye occurs within the fluid electronegative inclusions, the precipitates formed later in the fluid inclusions under the action of the dye are finally dissolved and give to these inclusions a homogeneous color. To explain this, VON MÖLLENDORFF supposes that the basic dyes penetrate the cytoplasm because of the lipides which it contains and in which the dyes are soluble. Then they accumulate in the previously formed acid inclusions. There, the mixture of two colloids of opposite signs, *i.e.*, the electropositive dye and the electronegative vacuolar colloid, produce precipitates. Then, when an excess of dye is found in the vacuolar colloid, it communicates its charge to the colloid, whereupon the precipitates are dissolved.

It seems justifiable to apply this reasoning to the staining of vacuoles of plant cells with vital dyes and we shall see, further on, that the work we have carried out on yeasts, in collaboration with GAUTHERET, seems indeed to confirm VON MÖLLENDORFF'S hypothesis. There is yet to be explained why the protoplasm remains uncolored. Neutral red has an oxidation-reduction potential which makes it unthinkable that it could be reduced in the cytoplasm, and it must be supposed that the dye traverses the cytoplasm in a degree of concentration which is too weak for its color to be perceptible, and then later accumulates in the vacuole.

The summary of vital staining which we have just given for the vacuoles of yeasts with a dilute colloidal content applies to a great number of cells, notably to the cells of the majority of fungi, but it is not altogether general. In other cells, vital staining operates in a different way. There are cases in which there is no production of precipitates in those vacuoles which from the beginning take a diffuse stain. There are other more frequent cases, in which both precipitates and a diffuse staining of the vacuolar sap occur at the same time. This diversity of behavior of the vacuoles seems to depend on the nature of the colloids which they contain and which are known to vary greatly from one type of cell to another.

It would seem today, in the light of investigations of BUNGENBERG DE JONG, that the precipitation observed in vacuoles under the influence of vital dyes may perhaps be explained, in many cases, not by flocculation, but by the production of coacervates in the vacuolar sap. According to this idea, there is a formation of a complex between the positive dye and the negative vacuolar colloid. The colloid may then flocculate as in the yeasts, or else, if the micelles are strongly bound to the dispersion medium, there is a separation of a coacervate phase in the vacuolar sap which, however, still contains dispersed colloidal micelles, as the diffuse color taken by the vacuolar sap indicates. If the dye continues to penetrate the vacuole, at first the coacervate phase becomes greater and greater. Then the micelles of the coacervate take the charge of the dye and, their repulsion becoming greater than their force of coherence, the coacervate disappears and the vacuole becomes uniformly stained. An observation which would seem to support this opinion further, is that the precipitates formed under the action of the dye, seem often to be in liquid droplets, capable of changing shape and even of becoming vacuolized. Moreover, it seems that all intermediary stages in the action of vital dyes between coacervation and flocculation can be found.

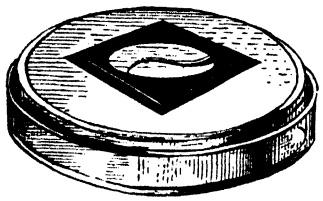


FIG. 88. — Petri dish designed for microscopic observation of living material.

Another point very clear from our investigations is that staining of the vacuoles by neutral red is *essentially a vital phenomenon.* As a matter of fact, although neutral red is only slightly toxic, if used in too strong concentrations, it may cause the death of the cell. This is preceded by an increase in the refractivity of the cytoplasm. Then suddenly the vacuole becomes colorless, whereas the entire protoplasm takes on a dark red color.

There is no formation of vacuoles or artificial granules preceding death in any of the cells which we have observed, and death is always accompanied by destaining of the vacuole. Staining of the vacuole is possible, therefore, only when the cell is living and this is a general fact which applies to the staining action of all vital dyes.

This fact may be even more satisfactorily observed in the lower algae, for instance in the Euglenas, which are endowed with movement. The cells of these algae accumulate neutral red in their vacuoles as long as they are moving but once their movements cease and the cells die, the vacuoles destain and the dye colors the cytoplasm and nucleus. The conclusion to be drawn is that the staining of the vacuoles in a cell constitutes one of the best means of determining if it is alive. Vital staining of the vacuoles and that of the chondriosomes and leucoplasts are therefore clearly differentiated. Vital staining of the last two is possible but when it occurs it is transitory. Usually it is sublethal and does not disappear when the cells die.

We have tried, by cultures of various plants in nutrient media to which neutral red has been added, to follow under the microscope the development of the vacuoles during cellular growth. This has, in addition, made it possible for us to judge the degree of toxicity of this dye. We have been able to obtain cultures of *Saprolegnia,* for example, on soy bean bouillon to which 0.001%-0.002% of neutral red or neutral violet have been added. The cultures were grown in Petri dishes (Fig. 88) whose bottoms had a 3 cm. opening covered by a cover slip sealed by asphalt cement (model used at the International Bureau for the Culture of Fungi at Baarn). It sufficed to turn the box over and place it under the microscope to be able to follow the development of the fungus under the oil immersion lens and to follow the life history of the vacuoles. Various species of *Saprolegnia* cultivated undere these conditions developed as well as those in the control cultures and followed out their entire life history from germination of the zoospores to the formation of zoosporangia. Now, during all their development the dye accumulated in their vacuoles and stained them superbly. In solutions of neutral red beginning with a concentration of 0.005%, growth is somewhat retarded. The fungus can stand relatively large doses of neutral red (0.05%-0.06%), although when the concentration of the dye is above a certain paint, it grows only very little.

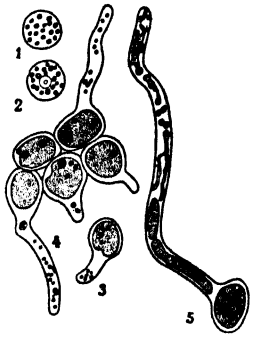


FIG. 89. — *Saprolegnia dioica.* Zoospores grown on gelatinized soy bean bouillon containing 0.001% neutral red. 1, before germination; vacuolar system consisting of numerous globular bodies stained by the dye. 2, id.; nucleus visible. 3, 4, fusion of small vacuoles into one large one containing granules precipitated by neutral red. 3, in the germination tube small, spherical, uniformly stained vacuoles are formed which in (5) elongate into filaments, form a network and then fuse to form large vacuoles.

With DUFRÉNOY and LABROUSSE, we were able to germinate seeds of tobacco in pure culture on Knop's solution to which had been added doses of neutral red from 0.005%-0.02%. We succeeded in doing the same with grains of wheat, barley and lupin seeds, in collaboration either with OBATON or with GAUTHERET. The seeds germinated normally under these conditions and by examining their roots in the Petri dish under a microscope it was possible to see that during the entire growth of the root, neutral red was accumulated in the vacuoles of the meristematic cells, in the differentiating cells of the cortex, in the root hairs and in the cells of the root cap. EICHHORN found also that roots of *Allium Cepa* grow perfectly in a solution of neutral red and show entirely normal mitoses. GAUTHERET for a period of several months kept alive the cells of the root cap of lupin in media to which neutral red had been added. The vacuoles in these cells were stained. In addition it may be recalled that SKUPIENSKI succeeded also in obtaining the complete development of one of the Myxomycetes, *Didymium nigripes,* in a medium to which neutral red was added. The vacuoles were stained during the entire growth of this plant.

This is, then, a valuable method of following under the microscope the entire life history of the vacuoles during cellular growth and we shall see further on the uses to which it may be put.

A fact becomes evident from these investigations, namely, that vital staining with neutral red is impossible if the medium is too acid. The starting point at which staining occurs lies between the *p*H values of 5.5 and 7, according to the type of cell in question. For roots of phanerogams it is, for example, 5.5, for the Saprolegniaceae 6.5, for yeasts and *Oidium lactis*, 7. This is an essential fact previously brought out by IRWIN and PISCHINGER whose work was later confirmed by BAILEY and ZIRKLE, GENAUD, CHADEFAUD and others.

It is curious to find that, with the exception of the Saprolegniaceae, most of the fungi are distinguished from all the other plants by their behavior in the presence of neutral red. Cultivated in media to which this dye has been added, they develop very readily but are never stained. We wondered why fungi which accumulate the dye so easily in their vacuoles when placed between slide and cover slip in a solution of neutral red, do not ever accumulate it while they are growing.

The species of *Saprolegnia*, in the conditions under which we cultivated them, never appreciably modify the *p*H value of the medium although other fungi make it more acid. The failure of the latter to be stained could therefore be attributed to a rapid acidification of the medium. This hypothesis, which we accepted at first and in which BECKER and SKUPIENSKI later concurred, is, however, not to be retained, for acidification of the medium may be considerably retarded and it is found that the fungi, even at a *p*H favorable to staining, do not take up neutral red. Recent research which we have carried out with GAUTHERET on *Oidium lactis* and various molds, has shown that these fungi accumulate neutral red only when they have ceased to grow. These same investigations also proved that when the spores of these fungi are sown in a medium to which neutral red has been added, they take up the dye, at first, and then become destained as soon as they begin to grow. If the cells of *Saccharomyces ellipsoideus* are sown, for example, in a moist chamber on an aqueous medium containing 1% peptone and 1% glucose at a *p*H of 7.5-8 to which has been added 0.005% neutral red and enough agar to hold them in place so that they can be found under the microscope and if the cells are examined microscopically, it is found that all the cells accumulate the dye in their vacuoles even between slide and cover glass. The accumulation is at its maximum at the end of half an hour, then, after about three hours (two hours for some yeasts), the cells lose their color and it is only then that they begin to bud. The loss of color is brought about by a process which is the converse of that by which staining was accomplished. The homogeneously stained vacuolar sap loses its color and there are seen to appear in the vacuoles, intensely stained granules which little by little lose their color and disappear (Fig. 90).

The oxidation-reduction potential of neutral red scarcely permits this destaining to be attributed to a reduction of the dye and various experiments seem to indicate that yeasts do not reduce neutral red. The destaining of the vacuoles can only be explained, therefore, by assuming a destruction of neutral red, or an excretion of it by the yeasts. The following experiment throws light on this problem. One half gram of *Saccharomyces cerevisiae* at a *p*H of 8 is sown in a big flask containing the medium described above to which 0.005% of neutral red has been added. A sample of the liquid is taken at regular intervals and centrifuged. The sediment is examined under the microscope each time and the concentration of neutral red of the liquid is measured by Meunier's photo-electric colorimeter. The experiment proved that, at the end of half an hour, all the cells accumulated neutral red in their vacuoles and that the concentration of the dye in the liquid was reduced from 0.005%-0.0015%. There is therefore a very great absorption of the dye by the yeast. At the end of half an hour, the vacuoles begin to lose their stain and the concentration of neutral red in the liquid again increases and finally at the end of an hour all the cells are destained and the concentration of the dye in the liquid has returned very nearly to its original amount. The vacuoles are then completely destained. Now, various experiments having shown that under these conditions there is no absorption by the membranes of the cells, one is obliged to conclude that the yeasts, after having accumulated the neutral red in their vacuoles, excrete it into the medium. So it is only when they are freed of the dye that they begin to bud.

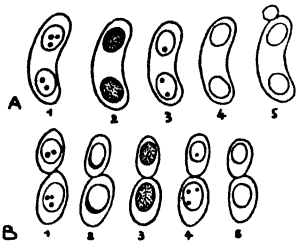


FIG. 90. — *Saccharomyces ellipsoideus* grown on 1% peptone and 1% glucose containing 0.005% neutral red. A. 1, First strongly colored precipitates are formed in the vacuoles; 2, The precipitates dissolve and the vacuole is diffusely stained; 4, The color disappears; 5, Budding. B. Similar phenomena. 1-3, staining; 4, 5, loss of color.

Saprolegnia behaves differently from the yeasts, since it accumulates neutral red while growing. Nevertheless it seems also to be able to excrete the dye under some conditions. In fact, although it always accumulates neutral red during growth, it does not keep it in the vacuoles unless the medium is poor in nutrients (soy bean bouillon with agar). If the culture is grown in a richer medium (peptone) it is found that, after having accumulated the neutral red at the beginning of growth, the cells suddenly lose their color at the end of 48 hours, *i.e.*, at the moment of maximum growth. From this point of view *Saprolegnia* occupies an intermediate position between the fungi on the one hand, which absorb neutral red only when their growth is arrested, and which reject the dye as soon as they begin to develop, and the phanerogams on the other hand, whose cells accumulate neutral red during their growth and retain it in their vacuoles during their entire development.

The other dyes which stain vacuoles, *i.e.*, cresyl blue, Nile blue, naphthylene blue and naphthylamine blue, behave a little differently. Between slide and cover glass, if the solution is too strong, they may, at the same time that they accumulate in the vacuoles, stain the cytoplasm and the nucleus with a diffuse color. This diffuse color occurs, furthermore, only in the phases which precede the death of the cells. At all events, with these dyes as with neutral red, the vacuoles destain and the staining of the cytoplasm and nucleus becomes accentuated as soon as death occurs. The coloration of the vacuoles by these dyes, as is the case for neutral red, is therefore possible only as long as the cell is living. It may be added that if the dyes are used in weak concentrations, in the fungi especially, they may be reduced in the cells and one sees the coloration disappear under the cover slip and reappear if the latter is raised for purposes of aeration.

We have also been able to germinate wheat grains in a medium to which these dyes had been added. Nile blue proved slightly more toxic than neutral red and neutral violet; cresyl blue is very appreciably more toxic than Nile blue; naphthylene blue and naphthyalmine blue are extremely toxic. These dyes are accumulated exclusively in the vacuoles of root cells, just as is neutral red, and brilliantly stain the vacuoles of meristematic cells, of root hairs and of cells of the root cap. The staining persists as long as the cells are in a living condition.

These dyes are more toxic for fungi. They do not generally accumulate in the vacuoles but are reduced by the Saprolegniaceae and by other fungi when cultivated in their presence.

In collaboration with GAUTHERET, some of the experiments on *Saccharomyces cerevisiae* that we carried out with neutral red were repeated, this time using Nile blue and cresyl blue. The experiments showed that these dyes behave like neutral red but have a much more complex action on the cells. These two dyes, Nile blue especially, may stain the cytoplasm. At a high *p*H, for example, Nile blue is at first retained exclusively by the cytoplasm which it stains diffusely. Then, at the end of a certain time, the dye is taken up by the vacuoles and from there excreted into the medium. When there is insufficient aeration, as is usually the case, the dye, held at first by the cytoplasm, is reduced by the cytoplasm to its leuco-derivative, in which form it goes into the vacuole and finally, still in its colorless state, is excreted into the external medium where it is re-oxidized in the presence of air.

Cresyl blue is only weakly retained by the cytoplasm and accumulates especially in the vacuole, but it may be reduced, as is Nile blue, before being excreted into the medium where it is re-oxidized.

Investigation of these dyes was carried further and the accumulation of neutral red, Nile blue, and cresyl blue by yeast cells was studied at different *p*H values. The method consists of sowing a known quantity of yeast (*S. cerevisiae*) in Schoen's medium (mineral salts, asparagine, glucose) to which 0.005% of the dye has been added at *p*H values scaling from 3-10. At the end of twenty

minutes, the liquid was centrifuged, the sediment examined under the microscope and the concentration of the dye in the liquid measured by the colorimeter. It was thus possible to plot in terms of *p*H the quantity of dye retained by the yeast. A study of the curves showed that the lower limit of *p*H at which accumulation of neutral red begins is much lower than 7. It is at *p*H 5.6. But at that point the staining of the vacuole is too weak to be visible under the microscope and only the measurements give evidence of the absorption of the dye. This is appreciable only beginning with a *p*H of 6.0. From a value of 5.6, the absorption gradually increases and the curve rises to a maximum at *p*H 7.6. From this point the curve descends. The quantity of neutral red absorbed at the maximum *p*H value is found to be 0.0042 g. for

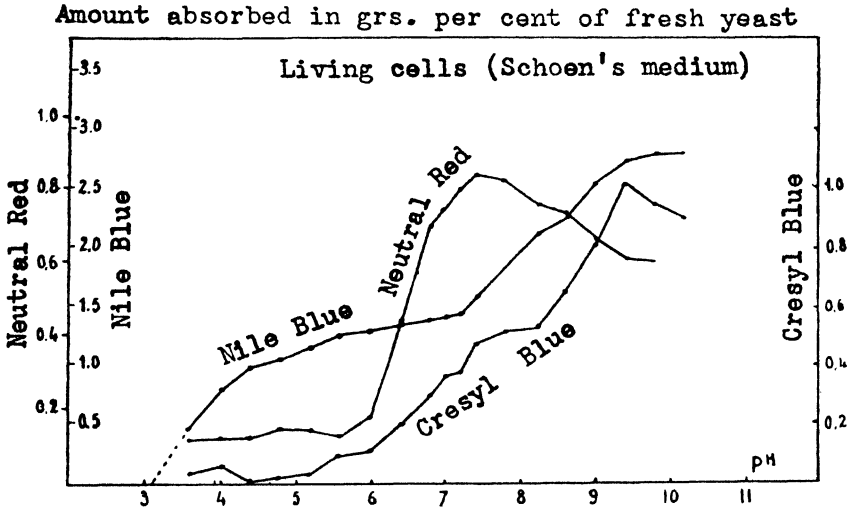


FIG. 91

0.5 g. of yeast (suspended in a 100 c.c. solution), which gives a concentration of 0.84 g. of neutral red for 100. g. of fresh yeast.

In the experiment with cresyl blue the lower limit at which accumulation of the dye begins is also at *p*H 5.6 but the curve does not redescend until about *p*H 10. The maximum quantity of cresyl blue absorbed is 0.0041 g. for 0.4 g. of yeast which makes a concentration of 1 g. of cresyl blue for 100 g. of fresh yeast. The curve for Nile blue is very different, for the lower limit is at *p*H 2.4 (absorption begins in a very acid medium) and steadily increases to *p*H 10, at which point the curve flattens out. The maximum quantity of Nile blue absorbed by 0.5 g. of yeast in the presence of 0.005 g. of Nile blue is 0.015 g. which makes a concentration of dye of 3 g. for 100 g. of fresh yeast.

These rather disconcerting results demand explanations. Two facts are indeed surprising: first the descending portion of the curve for neutral red after the maximum *p*H value is reached; sec-

ondly, the lower limit for accumulation of Nile blue placed at a *p*H value below 3 (2.4).

These apparent irregularities are easily interpreted. In studying the accumulation of neutral red by yeast, it is noticed that the quantity of dye retained by the cells varies incessantly, because of the alternate excretion and accumulation of the dye. There is not at any time, therefore, a true equilibrium and hence no real significance can be attributed to the absorption curve of neutral red in terms of *p*H values.

To obtain satisfactory results, a 1% solution of KH_2PO_4 must be used. In it the yeast does not grow and does not excrete the dye. Under these conditions, the amount of dye absorbed by the yeast varies very little and a state of equilibrium is reached which makes it possible to study accurately the accumulation of neutral red in terms of *p*H. The curve obtained in this medium is similar to that for cresyl blue. It begins at *p*H 5.6, rises steadily to *p*H 9 and then flattens out.

With regard to the second point, namely, the behavior for Nile blue, it must be taken into account that this dye is absorbed by the protoplasm of yeast and if the other dyes (neutral red and cresyl blue) have their lower limit of accumulation set at *p*H 5.6, one may think that the isoelectric point of their cytoplasmic colloids must be situated a little below this *p*H and that there occurs in its neighborhood a tightening of the micelles which prevents the penetration of these dyes, for they have not, like Nile blue, an affinity for the cytoplasm.

Our experiments showed, in addition, that the phenomenon of the accumulation of vital dyes in the vacuoles seems, up to a certain point, to follow the law of FREUNDLICH and to correspond to an adsorption[1].

The net result of these facts is that vital dyes, although not having any injurious effects on cells when used in weak doses, do behave as toxic substances and are always excreted into the vacuoles, where they remain (phanerogams), or from which they may be excreted into the exterior medium. The facts show us at the same time that the vacuoles are the centers of accumulation for toxic substances collected in the cells. These facts show that certain dyes may be taken up by the cytoplasm but that this is always transitory and the dye is very shortly excreted into the vacuole. It is only when the dye has been localized in the vacuole that the cell can grow and divide. There is an important difference, however, between the behavior in the phanerogams and in the fungi (*Saprolegnia* excepted). Whereas the phanerogam cells can grow when the dye has accumulated in the vacuole, those of the fungi can not and excrete the dye accumulated in the vacuole into the external medium. Once rid of the dye, the fungal cells begin to grow. Nevertheless our recent research (GUILLIERMOND and GAUTHERET)

[1] *Translator's note.* A fuller discussion of the entire subject is to be found in an article by GUILLIERMOND, received after this volume went to press: La Coloration vitale d'après des Travaux récents (Montpellier Médical 1940, No. 1:1-24).

minimizes this difference which formerly seemed inexplicable. Indeed, this work has shown that *Saprolegnia* which grows with the dye accumulated in its vacuoles can itself, in the course of development, excrete the dye from its vacuoles into the external medium under some conditions. This fungus, therefore, has a behavior intermediate between that of the phanerogams and that of the other fungi. It is not certain that the phanerogams themselves may not be capable of excreting the dye in their vacuoles to the exterior, but this is done with difficulty and under special conditions.

The purpose for exposing here the essential data of these still little known investigations before beginning the study of the vacuoles is to emphasize the following points. Among the dyes which stain the vacuoles, neutral red, Nile blue and cresyl blue are only very slightly toxic and, in general, bring about only a staining of the vacuoles. In addition, the staining of the vacuoles can be produced only during the life of the cell and is essentially a vital phenomenon. Furthermore, it is proved experimentally that neutral red is much the least toxic of these dyes and that it is never retained by the cytoplasm and, except in rare cases, accumulates only in the vacuoles. It is a stain which is *almost specific for vacuoles* and is the most useful dye for the study of these elements. It has, besides, the advantage of not being reduced by the cells, as are cresyl blue and Nile blue (in the case of the fungi). Neutral red, therefore, is indisputably the best vital stain for vacuoles.

This being the case, the importance of the method of vital staining in the study of the vacuoles is readily appreciated. This vital staining makes it possible not only to observe the vacuoles in preparations between slide and cover glass but also to follow, at least in most plants, the entire life history of these elements in cultures which are growing on media to which vital dyes have been added. This method of staining living material has led to important discoveries which we shall now set forth.

Chapter XIV

DEVELOPMENT OF THE VACUOLAR SYSTEM

First stages in development:- The starting point of the recent discoveries on the question of the development of the vacuolar system was an observation made by us on the mode of formation of anthocyanin pigment in teeth of leaflets from the rose bud. We noticed that the pigment begins to form at the extremity of the tooth. In examining a tooth from tip to base, all the phases in

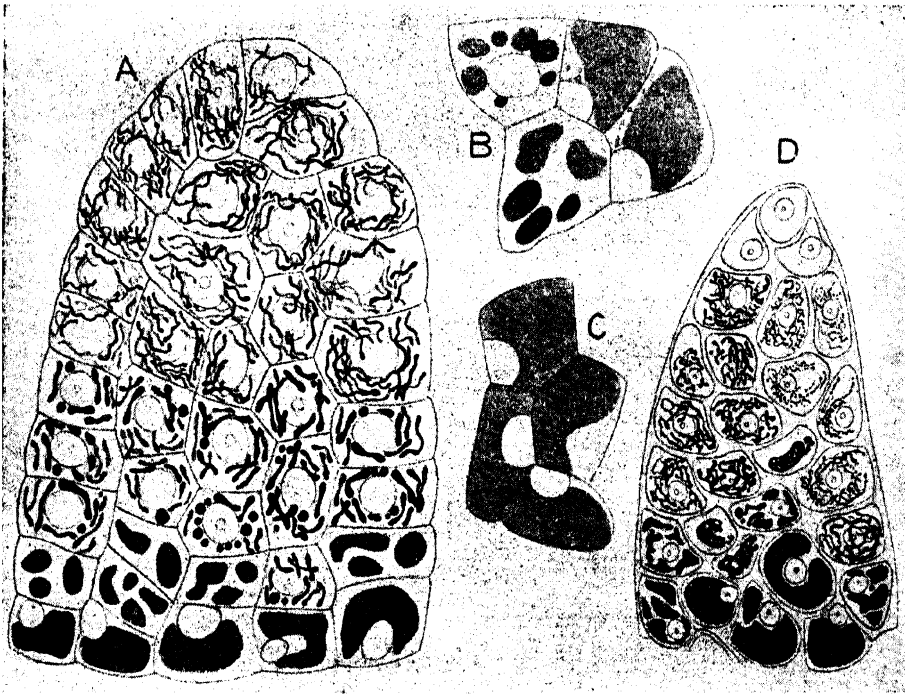


FIG. 92. — Teeth of young, living rose leaflets containing anthocyanin pigment which makes visible the vacuolar system developed from filamentous vacuoles which swell, anastomose and fuse to form one large vacuole per cell. A, D, cells at tip; B, C, older cells. D, after PENSA.

the formation of anthocyanin may be followed. Now, we observed that in the youngest cells, namely, those at the tip, the pigment appears as minute, numerous, filamentous elements very like the chondrioconts. These elements, taken all together, are exactly like a chondriome. In the region nearer the base, these elements seem to swell and be transformed gradually into small vacuoles, which, by their fusion, finally form a single enormous vacuole, occupying the major part of the cell and enclosing the

pigment in solution. By reason of the great resemblance between the initial shapes in which anthocyanin first appears and the shapes of the chondrioconts, we were led to think, originally, that this pigment arose in the elements of the chondriome which then became transformed into vacuoles. This interpretation was founded also on the fact that these shapes were preserved by mitochondrial techniques. With the method of Regaud, for example, we obtained at that time, both typical chondriosomes and elements of the same form as the chondriosomes, but a little larger, stained in the same way, but for which the staining was less stable and which, if destaining was prolonged, lost all the dye and took a yellow color. This color we attributed to the action of the potassium bichromate on the anthocyanin. Now, between the typical chondriosomes and these elements which resemble them, there seemed to exist all intermediate stages. We thought at that time, therefore, that the larger elements, to which the potassium bichromate gives a yellow color, corresponded to chondriosomes impregnated with anthocyanin. Our interpretation was a natural one at the moment, for the chondriome was not yet well known, the origin of the vacuoles was not understood, and it was thought that most of the pigments of animal cells were of mitochondrial origin.

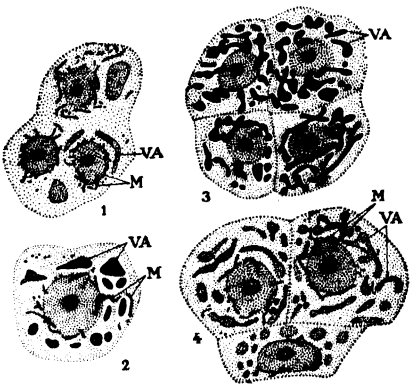


FIG. 93. — Oil glands of a walnut leaf. Regaud's method. 1, 4, preparations properly stained; chondriosomes (*M*) stained black, filamentous vacuoles (*VA*) containing an anthocyanin-tannin complex stained yellow brown. 2, 3, preparations insufficiently destained; 2, both elements black; 3, only vacuoles visible. Preparations such as 2, 3, might lead observers to think the chondriosomes are transformed into tannin-containing vacuoles.

This observation was immediately investigated by a certain number of workers among whom some agreed with our interpretations (MOREAU, MIRANDE) and others contested them. Among the latter ARTHUR MEYER thought, but without having proved it, that the chondriosome-like elements which mark the beginning of the formation of anthocyanin, represent filamentous vacuoles. LÖWSCHIN, on the other hand, expressed the opinion that these figures correspond merely to anthocyanin itself. According to him the anthocyanin is deposited in the cytoplasm in this form and consequently there is merely a fortuitous similarity in shape between these figures and that of the chondriosomes.

PENSA, stressing the fact that the chondriosome-shaped elements of anthocyanin always appear colored yellow by mitochondrial methods — probably in preparations too much destained — was led to think that they have no relation to the chondriosomes. Taking up LÖWSCHIN's theory and drawing upon his own observations, PENSA concluded that these figures correspond merely to an

aggregated state of anthocyanin and that this pigment might, according to the conditions in which the cell is at the time, assume two different colloidal states in the cytoplasm: an aggregated state characterized by the chondriosome-shaped elements scattered throughout the cytoplasm and a state of dispersion, *i.e.*, a state of pseudosolution in the cytoplasm. By treating with alkaloids those cells containing anthocyanin in the state of a pseudosolution in the cytoplasm, PENSA claims to have obtained a return of the pigments to the aggregated state, characterized by the production of anthocyanin granules assembled in little chains or in a network. Thus, according to PENSA, the chondriosome-shaped elements of anthocyanin do not always coincide with the stage of the formation of pigment. But PENSA's interpretation is erroneous, for the author did not understand that the pigment is dissolved in the vacuoles and not in the cytoplasm, and that the phases which he attributed to the state of pseudosolution of the pigment correspond to the dispersion state of the anthocyanin within a single enormous vacuole, occupying the major part of the cell and surrounded only by a thin layer of parietal cytoplasm. That which PENSA considers to be the cytoplasm is, therefore, nothing more than vacuolar sap. Alkaloids do indeed bring about flocculation (an aggregated state) of anthocyanin in the form of granules showing Brownian movement. The granules are precipitates of tannin absorbing the pigment as they form. These precipitates may assemble in little chains or in a network, and were erroneously likened by PENSA to the figures observed in meristematic cells. In the meristematic cells it is a question of small elements shaped like granules or filaments, resembling the chondriosomes, made up of a concentrated colloidal solution quite different from the vacuoles known at that time. DANGEARD later gave these small elements their true title of young vacuoles. There is nothing in common between these small vacuoles and the precipitations of anthocyanin obtained by PENSA in mature cells.

Credit must go to P. A. DANGEARD for having oriented this study in a new direction. We have already said that in studying the origin of the vacuoles in very diverse plants by means of vital stains, among others cresyl blue, this investigator found in the meristematic cells of plants and in the growing tips of fungal hyphae that vacuoles always appear as numerous and minute elements in the form of granules, isolated or united in little chains, or of filaments which often anastomose in networks, staining very deeply and homogeneously with vital dyes. These very closely resemble the chondriosomes and are composed of a very concentrated colloidal solution. They are elements which by hydration become transformed little by little in the course of cellular differentiation into fluid vacuoles.

Later, taking up our observations on the origin of anthocyanin, P. A. DANGEARD (*Cf.* p. 130) demonstrated that, just as ARTHUR MEYER had predicted, the elements which we had described as chondriosomes in reality represented young vacuoles with very

concentrated colloidal contents, the enclosed pigment giving them a natural color. Consequently, the formation of anthocyanin, from its beginning, is associated with the manner in which the vacuoles are generally formed. Struck by the resemblance between young vacuoles and the chondriosomes, as we ourselves had been, DANGEARD was led at the beginning of his research to liken them to chondriosomes and to think that the forms described under this name in animal cells corresponded to certain aspects of vacuolar development analogous to those forms encountered in plant cells.

He believed, furthermore, that he could demonstrate that the colloidal substance of which the vacuoles are formed, corresponded to a special substance found in all vacuoles, regardless of the type of cell, which he identified with *metachromatin* (*volutin* of MEYER). Consequently, in spite of current opinion, there could be no relation between the chondriosomes and the plastids. Hence DANGEARD gave the name *vacuome,* or *vacuolar system,* to all the vacuoles contained in a cell in the various phases of its development. This expression was destined, in his own mind, to replace that of chondriome and the term mitochondrium that of mitochondrial substance. "The greatest error of cytologists," he said, "is to have confused the chondriome and the metachromatin with the plastids. This, at any rate, is what I am going to try to demonstrate. The chondriome, which has been the object of so much investigation, must, in my opinion, be considered otherwise than it has been to the present time. It may be defined as the whole vacuolar system in its various and successive aspects."

Starting from the fact that vacuoles are stained by dyes and are capable of taking up almost the entire amount of dye in a solution, DANGEARD made his vacuome play an essential rôle in the phenomena of nutrition of the cell. According to him, metachromatin, the substance specific for the vacuome, plays at one and the same time an osmotic and a selective rôle. It fixes the nutrients as it, in turn, is fixed by the vital stains. In this way DANGEARD explains the formation of anthocyanin pigments. These, according to him, arise in the cytoplasm and are fixed by the metachromatin of the vacuome by virtue of its selective power. Thus DANGEARD transfers to the vacuome, the hypothesis which REGAUD had proposed to explain the rôle of the chondriome.

Lastly, P. A. DANGEARD and his son, P. DANGEARD, think that the vacuoles are permanent elements of the cell and multiply only by division, thus agreeing with the conception of DE VRIES, but with this difference that, for the DANGEARDS, the vacuoles by dehydration of the metachromatin may become solid in certain phases. This is true for aleurone grains and vacuoles of dormant spores of fungi.

This notion of taking the chondriome for the vacuome, which rests exclusively on observations made with vital stains, was inadmissible, it being true that at that time it had already been demonstrated that the chondriosomes are not stained by the dyes used by P. A. DANGEARD, but only, and then with difficulty, by

other dyes which do not have a predilection for the vacuoles. Moreover, the chondriosome-shaped vacuoles are of temporary nature and, even beginning with the first stages of cellular development, these vacuoles take in water and become transformed into large vacuoles, whereas it is known that the chondriosomes persist during the entire life of the cell. The only question that could be asked was whether, as we had supposed, the vacuoles were not derived in some cases from the chondriosomes. It was not logical to say that the chondriome and the vacuome were one and the same thing. The interpretation of DANGEARD has therefore been abandoned for a long time now, even apparently by its author, although without explicit statement to that effect.

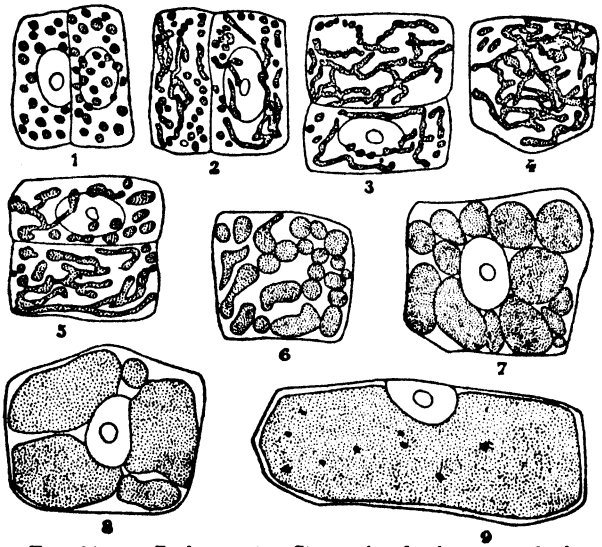


FIG. 94. — Barley root. Stages in development of the vacuolar system. 1-5, meristem; 6-8, adjacent region of differentiation; 9, mature cells of cortical parenchyma. Vital staining with neutral red.

Our investigations, beginning with that period, gave more details and indeed confirmed the observations of P. A. DANGEARD in so far as the development of the vacuoles is concerned, but they showed that the chondriosome-shaped vacuoles and the chondriosomes have in common only their shape, and that the vacuome is a system completely independent of the chondriome. They showed, furthermore, that the vacuoles are not characterized by a specific substance corresponding to metachromatin and that the colloids which the vacuoles contain are substances of very diverse nature, having as a common property only their ability to absorb vital stains. These facts were afterwards verified by a large number of cytologists and are today definitely accepted. The vacuoles do not, in general, stain by mitochondrial techniques and it is the very complex case of the leaflets of the rose which has caused all the difficulty. It has been seen that in the cells in the young teeth of

these leaflets (Fig. 92) there are chondriosome-shaped vacuoles containing a substance which, when preserved with mitochondrial methods, becomes yellow, but which may be stained by iron haematoxylin and which, if the destaining is not carried far enough, appears black. This would make one think that these bodies correspond to chondriosomes impregnated with anthocyanin. In reality, as we were able to demonstrate by later work, they are vacuoles containing tannin with which anthocyanin is associated. Now, tannin, like the lipide substance of the chondriosomes, is rendered insoluble with fixatives containing potassium bichromate, and may be stained by iron haematoxylin. On the other hand, the study of plants in which anthocyanin is not associated with tannin, makes it possible to observe that this pigment is not preserved by mitochondrial techniques and that the young chondriosome-shaped vacuoles in which pigment forms do not stain with mitochondrial methods. Therefore, DANGEARD, instead of correcting a partial error committed by us, made the mistake of generalizing it.

FIG. 95. — Young flower of *Iris germanica*. Early stages in the development of the vacuolar system in the trichomes of sepals. Vitally stained with neutral red.

Let us examine in more detail the development of the vacuoles in the phanerogams. In the barley root (Fig. 94), for example, the phenomena studied by DANGEARD and then by us, are particularly clear. If a very young root of a seedling is studied in a solution of neutral red by crushing the root gently, in such a way as to dissociate the cells without injuring them, numerous minute elements are seen in all the cells of the meristem. They are scattered about in the cytoplasm and are stained deeply and homogeneously by the dye. In the very youngest cells these elements are all small granules but they soon elongate to undulous filaments which often afterwards anastomose into a network. By their form and their dimensions these elements show a striking resemblance to the chondriosomes and an observer not forewarned would easily take them for such. Nevertheless, they are distinguishable at first sight from the chondriosomes by a much greater diversity of appearance and by the fact that they are reticulate. They seem to be composed of a very concentrated colloidal solution of semi-fluid consistency whose refractivity is such that they can easily be seen without vital staining.

In the course of cellular differentiation, it is observed that these elements swell by absorbing water and then coalesce. They are of

most diverse appearance: dumb-bell-shaped, granules arranged like a string of beads, club-shaped, spindle-shaped, networks of moniliform filaments. They then become transformed into small, spherical vacuoles which always stain uniformly, but as they continue to take in water, they soon appear only faintly colored. They sometimes contain a few deeply stained precipitates, which show Brownian movement and are caused by the precipitation of some of the colloidal contents of the vacuole under the influence of the dye. These vacuoles fuse and finally, in mature cells, form a single large vacuole which occupies the major part of the cell, pushing the nucleus and cytoplasm to the periphery. The cytoplasm is now reduced to a thin layer around the vacuole which appears faintly colored and shows only a few precipitates.

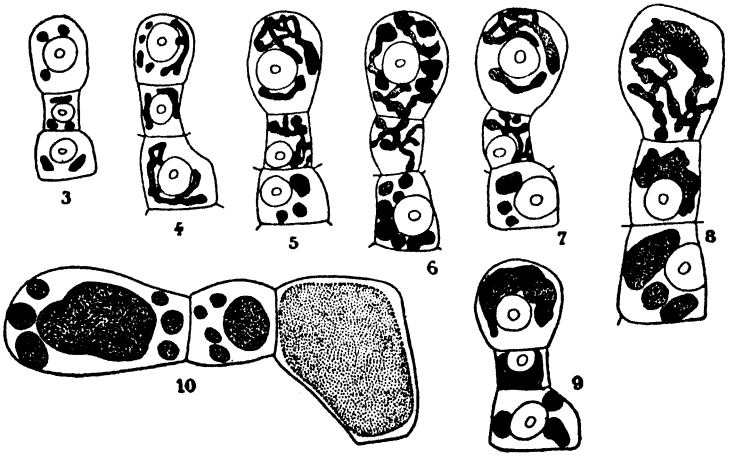


FIG. 96. — *Anagallis arvensis.* Stages in the formation of a glandular hair on the corolla; anthocyanin pigments present from the first (*in vivo*).

The development of the vacuolar system in the root of the wheat may be quite as easily observed and the phenomena take place in the same way. In most of the phanerogams and the pteridophytes[1], moreover, an analogous development of the vacuolar system is recognized. Excellent examples are furnished by the epidermal cells of very young leaves of *Iris germanica,* by the cells of very young hairs from the sepals of the same plant (Fig. 95), by the glandular hairs on the leaflets of the walnut (Fig. 93), by the leaves of *Anagallis arvensis* and others. In the glandular hairs, the vacuoles, like those in the teeth of the rose leaflets, contain from the very beginning an anthocyanin pigment which makes

[1] In the apical cell of the pteridophytes, however, there are found only large liquid vacuoles and in the cells of the meristem which are derived from the apical cell there are no small filamentous vacuoles (EMBERGER). It seems as if the apical cell had already passed through a stage in which the vacuoles were filamentous and semi-fluid, after which these filamentous vacuoles had taken in water and coalesced.

it possible to follow their entire development without using vital dyes. The large vacuoles of mature cells, instead of staining diffusely without precipitation or forming only a very few precipitates, may behave differently. Very often, as for example, in the epidermal cells of *Iris germanica,* the vital dyes do not stain the vacuolar sap and only produce in the vacuole deeply stained bodies showing Brownian movement or else bring about, at the same time, both a diffuse coloration of the vacuolar sap and the production in the sap of colored precipitates.

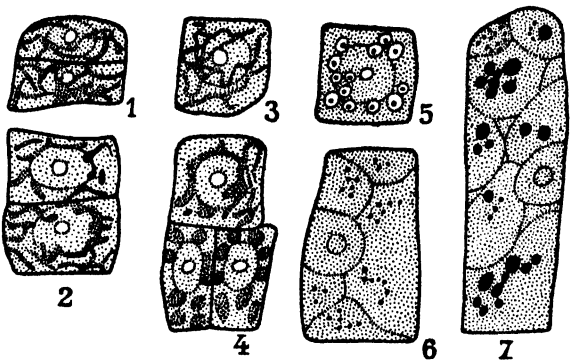


FIG. 97. — Pea root vitally stained with neutral red. 1-4, meristem; numerous small filamentous vacuoles, uniformly stained. 5-7, differentiating cells; swelling and fusion of small vacuoles, whose colloidal substance is precipitated by the dye as deeply stained bodies showing Brownian movement.

So the vacuoles seem to be at first small elements composed of a very concentrated colloidal solution which, by taking in water, gradually swell and coalesce during the period of differentiation of the cells, and become large vacuoles containing an extremely dilute colloidal solution. This progressive dilution of the vacuolar colloidal solution is made evident by the fact that neutral red, which at first gives the vacuoles a deep homogeneous color, stains them only weakly when the cells have attained a certain degree of dilution but generally brings about a precipitation of the colloid as deeply stained bodies which show Brownian movement. The precipitation is more or less copious depending upon the nature and concentration of the colloid.

When the vacuoles have come to the end of their development, they may, in the presence of vital stains, stain weakly and homogeneously without showing any, or at most very few, precipitates, as in the roots of barley and wheat (except for the cells of the root cap). In other, more frequent cases, vital dyes bring about the formation of numerous deeply stained precipitates, which show Brownian movement, at the same time that they stain the vacuolar sap diffusely (vacuoles in the epidermal cells of *Iris germanica*). In still other cases, the vital dyes at first cause only colored precipitates. These later fuse and may dissolve in the vacuolar sap which then becomes diffusely colored.

Rather often, there are normally found in the vacuoles more or less large, spherical bodies, or small granules united in mulberry-shaped masses, which are the result of a partial precipitation of the colloidal solution within the vacuoles. These bodies stain with vital dyes which, at the same time, bring about other precipitates of the same or of different natures. The normal presence of these bodies in the vacuole could be explained by the fact that the colloidal micelles contained in the vacuoles in some cases do not possess a power of unlimited imbibition, so a time seems to come when they cease to take in water. A disturbance in equilibrium occurs and this leads to the production in the vacuolar sap of a coacervate.

It may be added that rather frequently the large vacuoles of mature cells, especially when they contain tannins, continue to enclose a very concentrated solution and in living material are exceedingly refractive. These vacuoles which seem to be in the state of a jelly do not form precipitates with vital dyes or else form them with great difficulty. In the latter case they show an intense homogeneous color. There are even cases in which the jelly is almost solid and in such cases it becomes very difficult to plasmolyze the cells, as is seen in the vacuoles of the pericarp of *Ilex Aquifolium* (GUILLIERMOND, CHAZE).

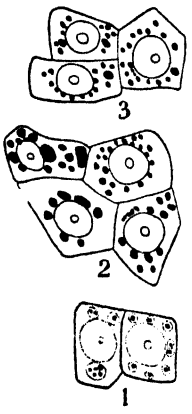


FIG. 98. — Bud of *Elodea canadensis* vitally stained with neutral red. Vacuoles globular and uniformly stained; or small and colorless with one or several colored precipitates.

Vacuoles behave differently according to the nature of their contents.

The development of the vacuolar system which we have just described is of very general occurrence and has been observed in very widely separated plants (phanerogams: P. A. DANGEARD, GUILLIERMOND, P. DANGEARD, BAILEY, ZIRKLE and others; pteridophytes: EMBERGER).

All the vacuoles of the higher plants however, do not follow exactly this development. Thus, in studying the formation of the vacuoles in the bud of *Elodea canadensis,* it is observed that in all the cells of the meristem of the stem and of the youngest foliar primordia, there are numerous, very small vacuoles which are always globose and never filamentous. These generally stain uniformly and deeply with neutral red, but in certain cases there is seen in their interior a single, deeply stained, corpuscle showing Brownian movement. This corpuscle has been produced by a precipitation of the colloidal contents of the vacuole. These vacuoles, which are not visible in living material without being stained, seem to be constituted of a less concentrated colloidal solution than are those in ordinary cases. They later swell up by taking in water and then gradually coalesce to form, in mature cells, a single vacuole which neutral red stains diffusely while also causing the production of numerous precipitates. There are, therefore, no filamentous or reticulate formations

but only spherical vacuoles to be found at the beginning of development of this type of vacuolar system.

Among the fungi, the Saprolegniaceae constitute particularly favorable objects for the study of the vacuolar system, to which we will have to return later. When the mycelium of a species of *Saprolegnia* is examined in a solution of neutral red, there are observed at the growing extremities of the plant, vacuoles which at first are generally small globular elements. These elongate and appear as thin, tenuous canaliculi, more or less oriented in the direction of the longitudinal axis of the hypha. These canaliculi anastomose and form a delicate and complicated network. In this region they appear more fluid and with too little difference in refractivity be-

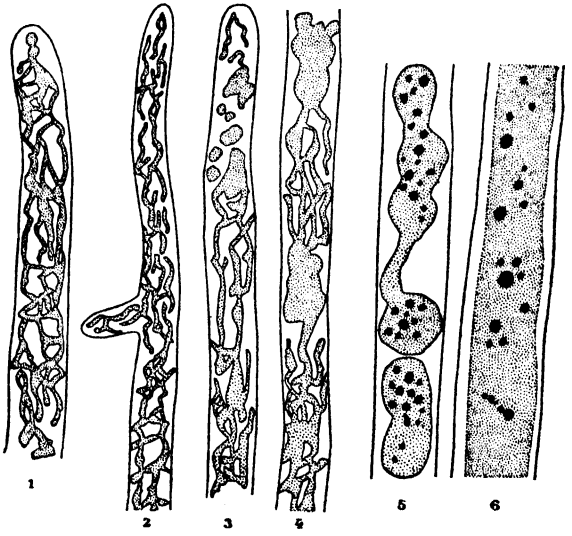


FIG. 99. — *Saprolegnia* vitally stained with neutral red. Development of the vacuolar system. 1-3, tips of growing filaments; anastomosing canaliculi. 4, older filaments; fusion and swelling of canaliculi. 5, later stage; precipitated bodies in sap. 6, still later stage; single canal containing precipitates.

tween them and the cytoplasm for them to be observed without staining. A little further from the tip of the hyphae, these canaliculi are observed to swell, little by little, then to converge, so that in differentiated regions of the hyphae, there is only a single canal running from one end of the siphon to the other. This canal occupies the major part of the hyphae and in it the vital dyes produce both numerous, semi-fluid, precipitates and diffuse staining of the vacuolar sap. The cytoplasm, accordingly, now constitutes only a thin layer about this canal and the nuclei are appressed to the wall of the siphon.

This vacuole, therefore, is of a special type; namely, a single canal running from one end of the filament to the other and obviously adapted to the siphonate structure of the Saprolegniaceae. This form can be found in *Vaucheria* but it is a rather rare type.

In most fungi, except for the Phycomycetes, the vacuoles appear in the tips of the growing hyphae as very numerous, small, globular elements which sometimes stain uniformly and deeply with the vital dyes and sometimes remain uncolored but contain a deeply stained corpuscle showing Brownian movement (Fig. 100). These vacuoles swell in regions farther away from the tip, then coalesce, until, in the regions still farther away, they form large colorless vacuoles filled with deeply stained corpuscles. These bodies swell after a time, then may dissolve and give the vacuole a diffuse and homogeneous color. This is also true in the yeasts in which there exist in the bud several small, spherical vacuoles, sometimes uniformly stained with neutral red, sometimes not stained at all, but containing colored corpuscles. These small vacuoles fuse during the growth of the bud until there is present only one large vacuole or a few large vacuoles filled with stained corpuscles showing Brownian movement. (Fig. 101).

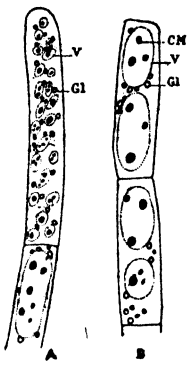


FIG. 100. — *Endomyces Magnusii.* Vital staining with neutral red of filaments whose vacuoles (*V*) contain intensely colored granules (*CM*) which show Brownian movement and which correspond to bodies of metachromatin obtained with Bouin's fixative stained with haematein. *Gl*, lipide granules around vacuoles.

In some of the lower plants, the vacuoles develop quite differently. In many algae, the Conjugatae for example, they are always in the form of large liquid vacuoles. In other algae they may, on the contrary, appear during the entire cellular development as very small, semi-fluid, usually globular, vacuoles, scattered about in the cytoplasm and never undergoing hydration. These vacuoles, formed of a very concentrated colloidal solution, stain homogeneously and deeply with neutral red, usually without precipitation. This is the type of vacuole generally found in the Phytoflagellates (Euglenas, Peridinieae and Volvocales observed by the DANGEARDS), in certain land forms of the Chlorophyceae (*Pleurococcus, Pleurastrum, Prasiola* reported by PUYMALY). The Cyanophyceae also contain similar vacuoles which are found localized in the parietal cytoplasmic layer surrounding the central body, *i.e.*, the region which corresponds to the nucleus (GUILLIERMOND). The bacteria seem to belong to this category. In them, by vital staining or after fixation, there are observed, especially at the poles of the cell, metachromatic corpuscles which seem to correspond to small vacuoles with very concentrated metachromatin (GUILLIERMOND, Mlle. DELAPORTE). Vacuoles of this type are encountered in the conidia, in the spores and in the zoospores, of fungi and algae (zoospores of Saprolegniaceae and *Ulothrix* (Fig. 103), for example), and in pollen grains (P. DANGEARD, Mlle. PY).[1]

The data which we have just reviewed, confirm, therefore, the observations of P. A. DANGEARD. These data show that vacuoles

[1]Mlle. PY has shown that in dehydrating pollen in a vacuum, a solidification of these vacuoles is obtained. The vacuoles become comparable to aleurone grains within the pollen grains, although the pollen grains do not lose their viability.

seem to be encountered in all cells in all phases of development and that, in general, they appear in embryonic cells as minute elements composed of a very concentrated colloidal solution and that they sometimes show a great resemblance in form and dimensions to the chondriosomes. These chondriosome-shaped elements swell and are transformed into large liquid vacuoles containing very dilute colloidal solutions. These facts do not, however, confirm DANGEARD'S interpretation in which he identifies the vacuolar system with the chondriome. Although in some cells the young vacuoles present forms almost identical with those of the chondriosomes, there are other very numerous cases in which the young vacuoles, on the contrary, have an appearance which does not permit of any confusion with the chondriosomes. For example, in some algae, the vacuoles remain constantly in the state of large liquid inclusions. Nevertheless, since it is evident that these two categories of elements may sometimes be easily mistaken for one another, it is advisable to examine here the characteristics which make it possible to distinguish between them.

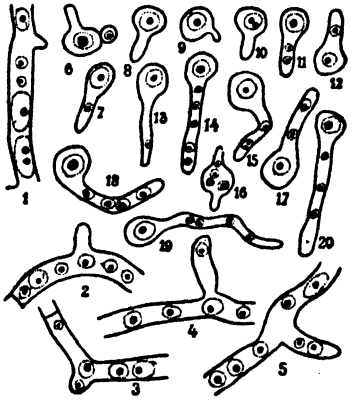


FIG. 101. — *Penicillium glaucum.* Vital staining with neutral red. 1-5, filaments; one or more large, deeply stained bodies precipitated in the vacuole by the dye; small vacuoles in branches seen to form *de novo*. 6-19, germinating conidia; small vacuoles in germination tube seem to form *de novo*.

Chondriosome-shaped vacuoles and chondriosomes. Characteristic differences:- The vacuoles, even in their chondriosome-shaped state, are essentially distinct from the chondriosomes in their histochemical characteristics. An inherent difference rests in the fact that although the vacuoles stain deeply with vital stains (neutral red, cresyl blue, Nile blue, etc.), the chondriosomes, on the contrary, have no affinity for these dyes and are not colored in the living state except with special stains (Janus green, Dahlia violet, methyl violet), which usually show no marked affinity for the vacuoles. Besides, as has been seen, staining of the vacuoles is essentially a vital phenomenon and ceases when the cells are killed. On the contrary, the chondriosomes stain only temporarily; their coloration is stable only in dying cells and is then always accompanied by vesiculation, a state soon followed by the death of the cells. The coloration persists, even after the death of the cell. Meristematic cells may be found in which chondriosomes are visible in the living state and the independence of these from the vacuoles may be made certain by vital staining, since the chondriosomes remain unstained. The same observation may be made for the Saprolegniaceae in which the chondriosomes are always clearly visible in the living state in all stages of development of the plant. We have obtained, furthermore, in these and other fungi (*Endo-*

myces Magnusii) a double vital staining of the chondriome and the vacuolar system by means of using a mixture of a solution of neutral red and Janus green or Dahlia violet. In this way, we were able to follow the simultaneous development of the two systems during the entire growth of the plant. The chondriosomes by this method are colored green or blue and the vacuoles red. (Fig. 104).

These initial forms of the vacuoles which look like chondriosomes, are very fragile, just as the chondriosomes are, and they swell and fuse into larger spherical vacuoles during prolonged observations with vital staining, but this alteration of shape has nothing in common with the cavulation of the chondriosomes (*cf.* p. 101). Solutions of osmic acid preserve and heavily blacken the chondriosome-shaped vacuoles whenever they contain phenolic compounds; otherwise it may preserve them in a greatly swollen condition without blackening them. It is known that the chondriosomes are, on the contrary, very well preserved with osmic acid but are not darkened by it.

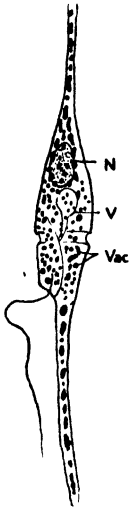


FIG. 102. — *Ceratium strictum*. *N*, nucleus; *V*, vesicle; *Vac*, vacuoles. (After DANGEARD).

In preparations treated by mitochondrial techniques, the chondriosome-shaped vacuoles usually appear as uncolored canaliculi, sometimes anastomosing in a network in the midst of the faintly colored cytoplasm. Sometimes the contents of the vacuole are colored, but this is rare. When it does occur, however, they are always found condensed by the action of the fixatives in the middle of the colorless canaliculi so that it is not possible to confuse the elements of the vacuolar system with the chondriosomes. Sometimes also the colloidal contents of large liquid vacuoles, derived from the chondriosome-shaped vacuoles by hydration, show bodies precipitated by the action of the fixatives which stain with mitochondrial technique. (Figs. 105, 106).

In all cases in which the vacuoles contain tannins, the chondriosome-shaped vacuoles appear as filaments, or as a network, somewhat dilated by fixatives and colored yellow by potassium bichromate, or blackened by osmic acid, according to the method employed. The large liquid vacuoles resulting from their fusion show, with the same methods, either granular precipitations or large corpuscles stained yellow by potassium bichromate or blackened by osmic acid. But there is no method, in most cases, which makes it possible to stain the colloidal contents of the vacuoles after fixation, when they do not contain tannins, unless, perhaps, the Golgi methods which will be discussed later (Fig. 107 and 108).

In most fungi and some algae, however, we have seen that the colloidal substance of the vacuoles, or the metachromatin, made insoluble and precipitated in the form of corpuscles by formalin

or alcohol, is stained deeply red by aniline blue or violet basic dyes, as well as by haematein. It consequently shows a whole series of histochemical reactions which are very well known and are very different from those of the chondriosomes.

It is, therefore, well established that there does not exist the slightest relation between the chondriome and the vacuolar system; they are two independent systems which are coexistent in the cell. There is between the young stages of the vacuoles and the chondriosomes merely *a coincidence of form.* This close resemblance exists, however, only in a very limited phase in the development of the vacuoles and seems to be explained by the fact that the vacuoles are at that moment in a semi-fluid physical state which is closely allied to the physical state of the chondriosomes.

Physical characteristics of the vacuoles:- The chondriosome-shaped vacuoles seem to be of semi-fluid consistency and in a physical state which approaches that of the chondriosomes. Ultramicroscopic examination shows that in general the chondriosome-shaped vacuoles are no more visible than the chondriosomes (Fig. 109). In certain cases, however (teeth of young rose leaflets, barley and wheat roots), it is possible to distinguish them. They appear optically empty and are visible only because of their faintly luminous contours, *i.e.,* when they can be seen, they show the same characteristics in this respect as do the chondriosomes and the plastids. With the chondriosomes and the plastids, they might be classified as a coacervate system.

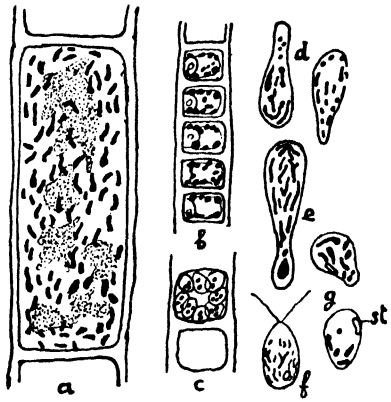


FIG. 103. — *a, Cladophora;* vital staining of large central and small peripheral vacuoles. *b, c, Ulothrix pseudo-flocca;* arrangement of vacuoles in *b* vegetative cells and in *c* zoosporangium. *d, e, Cladophora;* zoospores which have ceased swarming. *f, Bryopsis plumosa;* zoospore. *g, Ulva Lactuca;* zoospore which has just come to rest. *st,* stigma. (After DANGEARD).

The liquid vacuoles, derived by swelling from these chondriosome-shaped vacuoles, also appear optically empty even in cases in which they enclose an abundance of colloidal substances (tannin, metachromatin). Rather infrequently they are visible by reason of their faintly luminous contours. Certain indirect methods, however, often make it possible to locate their position. Thus in the yeasts, there exist in the cytoplasm bordering on the vacuoles, numerous lipide droplets which appear very luminous and because of them the position of the vacuoles may be easily detected (Fig. 110). There sometimes appear within the vacuoles strongly lighted granules which show Brownian movement, but these are always visible in direct lighting and are not of the order of micelles. The vacuole, then, in its liquid state seems to be composed either of a colloidal solution whose micelles are very small and not

visible, or of a very fluid hydrogel. Nevertheless this solution is very unstable and easily precipitable, as we have seen in studying the action of vital dyes on the vacuoles. Sometimes, however, the large vacuoles of mature cells remain, as has been said, in the state of a very concentrated colloidal solution, a sort of jelly. In this case, they are generally visible in the ultramicroscope because of their luminous contours.

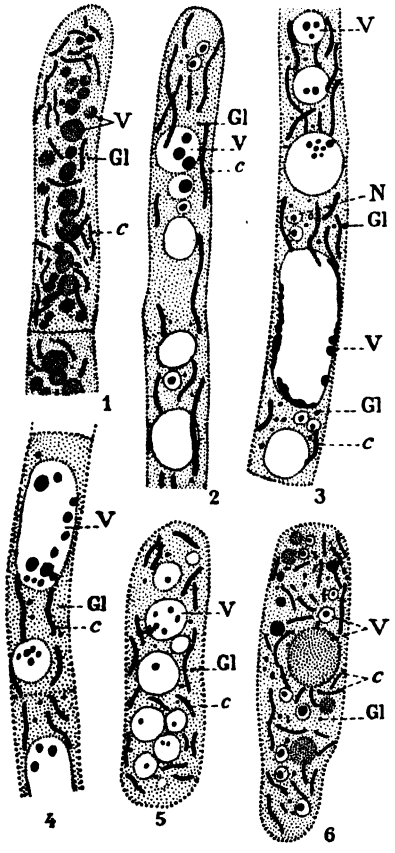


FIG. 104. — Filaments and oidia of *Endomyces Magnusii*. Double vital staining with Dahlia violet, which stains the chondriosomes (*c*), and with neutral red, which colors the young vacuoles (1, *V*), or in older vacuoles (*V*) causes precipitation of their metachromatic substances as deeply colored bodies. *Gl*, lipide granules.

The filamentous vacuoles seem, like the chondriosomes, to have a specific weight rather like that of the cytoplasm, although often it is higher. For example, ÅKERMAN found by use of the centrifuge that the filamentous vacuoles existing under certain conditions in the tentacles of *Drosera* are heavier than the cytoplasm. H. CLÉMENT, by the same process, was not able to displace the chondriosome-shaped vacuoles containing anthocyanin in the teeth of young rose leaflets. In recent work, MILOVIDOV has shown that, in the youngest cells of the teeth, the chondriosome-shaped vacuoles containing concentrated solutions of tannin and anthocyanin become oriented in the direction of the centrifugal force and are therefore heavier than cytoplasm. The large vacuoles derived from them, and containing a more dilute solution of tannin, become oriented in the opposite direction, *i.e.*, centripetally, and are lighter than the cytoplasm. MILOVIDOV obtained similar results with barley roots. In cells of the youngest part of the meristem, the vacuoles are heavier than the cytoplasm and are easily displaced in the direction of centrifugal force. In regions situated just a little above, the vacuoles which are beginning to take in water have about the same density as the cytoplasm and are no longer displaced. In the region in which the cells are already differentiated, the vacuoles are much lighter than the cytoplasm and are displaced in a centripetal direction. (Fig. 111).

Thus the small vacuoles which look like the chondriosomes are semi-fluid or sometimes almost solid elements. They seem to be

composed of a jelly or of a coacervate. One is therefore led to believe that the development of the vacuoles described in the preceding pages may possibly be nothing more than an unlimited imbibition of small elements, shaped like granules or filaments, which are in a jellied or coacervate state. This imbibition would involve the transformation of the original gel into a very dilute solution represented by the liquid vacuoles.

Chemical nature of the colloidal substance of vacuoles:- The histochemical characteristics of young vacuoles, which we have enumerated to establish a distinction between the chondriosome-shaped vacuoles and the chondriosomes themselves, prove that the

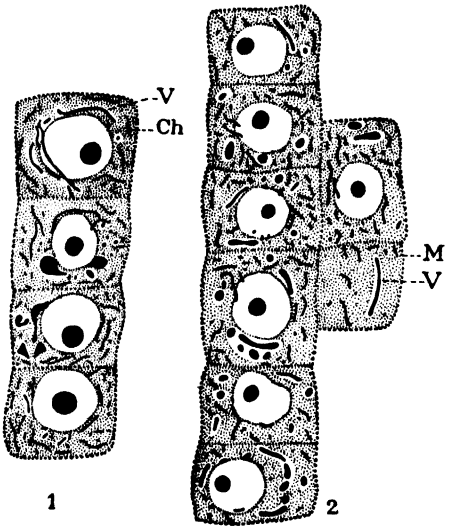


FIG. 105. — *Ricinus* root. Meristem fixed by Regaud's method. Black, filamentous or granular chondriosomes (*Ch*) and vacuoles (*V*), the latter distinguished by an external hyaline region due to a contraction of their colloidal contents during fixation.

colloidal substances of the vacuolar sap have an essentially variable constitution. Vacuoles are present in all plants but there does not exist any substance characteristic of them, as is the case for the chondriosomes. They contain diverse substances having nothing in common except their property of fixing vital stains.

Vital staining alone reveals differences in coloration between vacuoles. Cresyl blue, for example, changes color in vacuoles which contain metachromatin (majority of fungi, certain algae). It stains them a diffuse red and the enclosed corpuscles are colored dark red. In the cells of phanerogams, the staining of the vacuoles is extremely variable. Whenever the vacuoles contain phenolic compounds, they take a pure blue color with cresyl blue because of their acid *p*H. This same blue color is sometimes observed when the vacuoles contain lipide substances (phytosterol or phos-

pholipides reported by REILHES). In other cases the vacuoles take on colors toward the violet.

DANGEARD used the characteristic shown by blue vital dyes, of staining red or violet those vacuoles which contain metachromatin, as the only basis for his theory of the universal presence of metachromatin in vacuoles. But this staining reaction (Fr. *métachromasie*) is not a characteristic belonging alone to the substance called metachromatin. Metachromatin has been so named because it

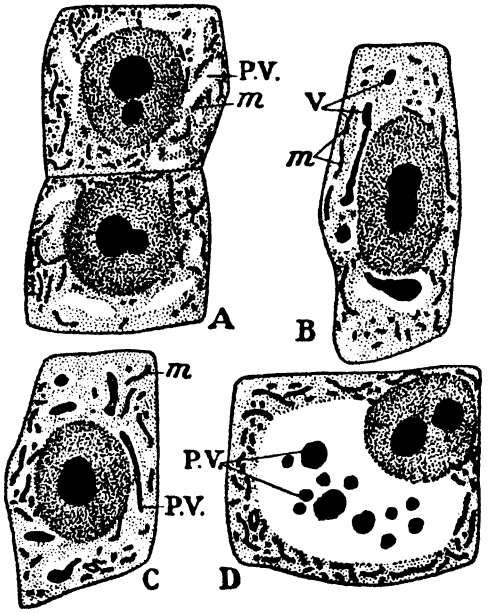


FIG. 106. — Root of pea. A, meristem; chondriosomes (*m*) stained, vacuoles (*P.V.*) colorless. B, C, meristem of central cylinder; contents of filamentous vacuoles (*P.V.*) clearly distinguished from the chondriosomes by a peripheral hyaline region caused by contraction of the contents of the vacuole by the fixative. D, differentiated cortical parenchyma; single large vacuole with densely stained bodies (*P.V.*). *m*, chondriosomes. *V*, vacuole. Regaud's method.

changes all blue and violet aniline dyes and haematein to a red color after fixation. Now this still unexplained phenomenon has no relation to the color change obtained with vital staining. One can not, as do the DANGEARDS, relate the colloidal contents of all vacuoles to a single substance and call it metachromatin, since metachromatin is a substance present in fungi, so named by reason of a color change which it shows after fixation, not when vitally stained. Besides, it has been seen that metachromatin is preserved by alcohol and formol, whereas the colloidal substance of the vacuoles is usually destroyed by all fixatives, even those of mitochondrial techniques.

If the blue coloration which the vacuoles sometimes take with cresyl blue is often an index of an acid reaction, as is the case for

the vacuoles containing phenolic compounds, the red or violet coloration which they take in other cases is difficult to explain, for it is known that cresyl blue in solution in pure water shows only a single change in color: it takes on an orange tint for a *p*H value of 11.2. Nevertheless, according to MANGENOT and Mlle LAURENT, cresyl blue at a *p*H which is not accurately measured, shows a change to violet, on condition that the medium contain diverse colloidal substances (sodium silicate, dextrine, protein, etc.) or even sugar (saccharose, according to unpublished work). This fact has been confirmed by CHADEFAUD.

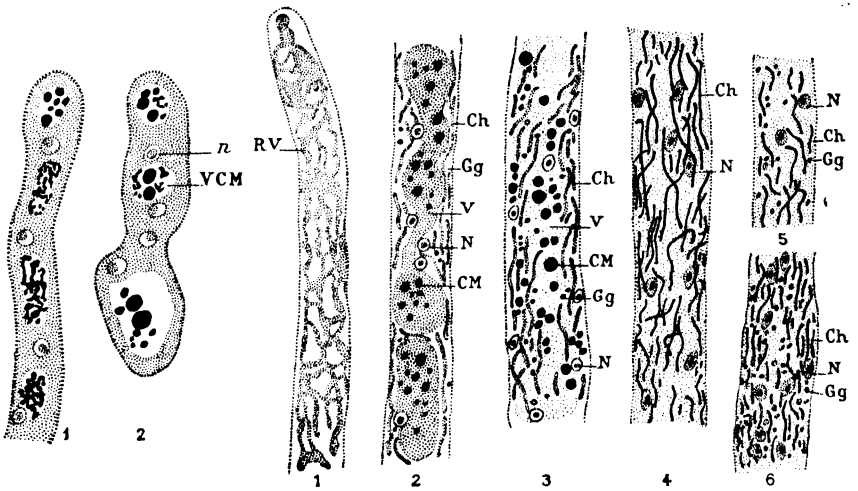


FIG. 107 (left). — *Dematium*. Bouin's method, stained with hemalum. 1, filament. 2, germinating conidium. *VCM*, vacuole containing metachromatin precipitates. *n*, nucleus.

FIG. 108 (right). — *Saprolegnia*. Chondriome and vacuolar system. 1-3, Vital staining with neutral red. 1, tip of filament; reticular vacuole (*RV*); other elements omitted. 2, older filament; tendency of network to become a diffusely stained canal (*V*) containing deeply stained bodies (*CM*), other elements visible but unstained. 3, still older filament; vacuolar canal (*V*) loses its stain, other bodies as in (2). 4, mature filaments. Regaud's method with iron haematoxylin; vacuolar canal unstained, lipide granules dissolved, chondriosomes (*Ch*) strongly stained. 5, 6, mature filament. Meves' method with acid fuchsin; chondriosomes (*Ch*) red, lipide granules (*Gg*) brown. *Ch*, chondriosomes. *N*, nucleus.

It must be added that the change to red in the vacuoles may also depend on the chemical constitution of colloidal substances which the vacuoles hold in solution. Mucilaginous substances, agar, for instance, change cresyl blue to a color toward the red, no matter what the *p*H. According to LISON, this color change in vital staining is a histochemical reaction characteristic of all sulfuric esters of high molecular weight. Now, mucilages or polyholoside esters are the most important among them.

From these considerations it follows, therefore, that the change toward red of the vacuoles, which is due to the most variable causes, and which may be obtained *in vitro* by the addition of the most diverse substances, can not possibly serve to characterize a chemical substance.

In general, the vacuolar colloids appear in the higher plants to be protein substances, perhaps proteins soluble in alcohol, and

are often associated or combined with tannins and perhaps with mucilages. LLOYD and various other authors have shown that tannin is often combined with mucilages in the state of a complex, and it is thought that the vacuoles containing raphides enclose mucilages. Furthermore, recent work has shown that in certain cells the vacuoles contain a colloidal solution of phytosterol or of phosphoaminolipides. These substances may become partially solid in the epidermal cells of the Liliaceae (Fig. 112). These cells ordinarily contain a large inclusion, formed of a complex of phytosterol and of phosphoaminolipides, which MIRANDE described for the first time under the name of *sterinoplast*. This, he considered

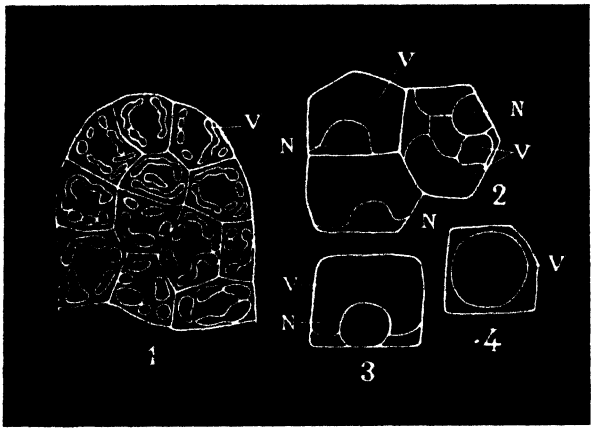


FIG. 109. — Rose. Cells from a tooth of a leaflet under the ultramicroscope, showing faintly luminous contours of the vacuoles (*V*) and of the nuclei (*N*). 1, tip of tooth. 2-4, differentiated cells.

to be a cytoplasmic inclusion, a sort of plastid, elaborating phytosterol. The work of MIRATON and of EMBERGER has demonstrated that the sterinoplasts are not simple vacuolar concretions. The recent work of REILHES has established the fact that the vacuolar sap of these cells contains a solution of phosphoaminolipides and of phytosterol which, in mature cells, becomes partially solidified in the vacuoles in the form of large bodies composed of a phosphoaminolipide-phytosterol complex. Inclusions, apparently of the same nature, have been cited in the vacuoles by other authors: in the epidermis of *Iris*, especially, in which they absorb anthocyanin, when the vacuoles contain the pigment, and have been described under the misnomer of *cyanoplasts* (POLITIS). Similar inclusions have also been described in the epidermis of the flowers of *Delphinium cultorum* (SCHARINGER). We have shown as well that in the cells of the root cap of barley and of wheat, there are phosphoaminolipide solidifications and, by cultivating barley roots in media very rich in sugar, GAUTHERET succeeded in making a great number of lipide concretions appear in the vacuoles of most of the cells. The vacuoles of *Monotropa* according to WEBER contain large

quantities of lipides. Furthermore, BUVAT reported in the vacuoles of many roots, the existence of phosphoaminolipide concretions sometimes combined with proteins.

In the algae, the colloidal substances of the vacuoles are also very diverse. Proteins, tannins and mucilages seem to exist in them but *metachromatin* and *volutin* are also frequently found. This last substance, which is also found in the bacteria, especially characterizes the fungi, in which, with the exception of some of

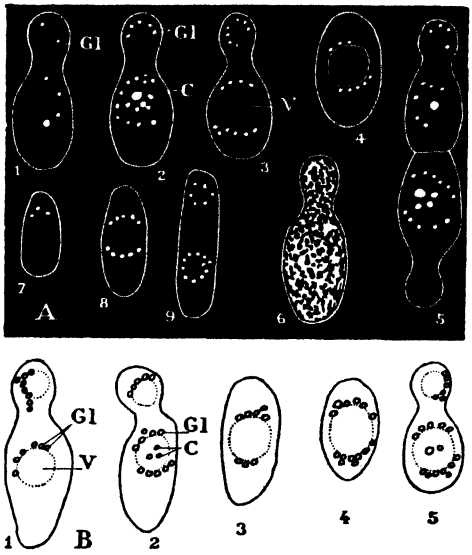


FIG. 110. — *Saccharomycodes.* (A), under the ultramicroscope; (B), with direct light. A, 1-6 *S. Ludwigii.* Vacuoles (*V*) usually invisible; position marked by strongly lighted lipide granules (*Gl;* A3, B5) surrounding them and corpuscles of metachromatin (*C;* A2, B2) which they contain. 4, contour of vacuole visible. 6, cytoplasm coagulated. 7-9, *S. pastorianus.* B, *S. Ludwigii.*

the Phycomycetes (Saprolegniaceae and Peronosporaceae), its presence is of general occurrence and in which it appears to play the rôle of reserve product. In fact this substance accumulates in the vacuoles of the epiplasm of the asci of yeasts and of the higher Ascomycetes and is absorbed by the ascospores at the same time as the glycogen and the lipides which are coexistent with it in the epiplasm. Volutin offers histochemical characteristics which as we have seen make recognition of it easy. Its chemical constitution is still not well determined but there are good reasons for supposing that it is formed by a combination with nucleic acid (ARTHUR MEYER).

From very recent work of Mlle. DELAPORTE and Mlle. ROUKELMAN on yeasts, it seems that metachromatin corresponds to a zymonucleic acid compound which has been extracted in great quantities in yeasts. According to these investigators, the nuclei of these fungi, as well as those of other plants probably, are com-

posed of thymonucleic acid, like those of animal cells, which explains why the Feulgen reaction is obtained just as well in plant, as in animal, nuclei. In the Saprolegniaceae, finally, we have found sphaerocrystals which appear to have some relation to the phosphoaminolipides.

The vacuoles contain, as well as the colloidal material just discussed, numerous crystalloid substances. The most wide-spread of these are organic acids, halogen salts, nitrates and phosphates, sugars (saccharose, glucose, fructose, etc.), heterosides and pigments. Among the halogen salts we must mention the presence of iodide in a dissolved state in the vacuolar sap of numerous marine algae (Rhodophyceae and Phaeophyceae). Its localization in the vacuole may be demonstrated *in vivo* by cresyl blue. This pigment in the presence of iodized solutions forms red crystals (Fig. 113) arranged in the shape of a bouquet (SAUVAGEAU and MANGENOT). Among the pigments may be mentioned the oxyflavanol pigments which are very pale yellow in color and the anthocyanin pigments which are red, violet, or blue. Both types are extremely widespread in green plants. Anthocyanin pigments, when found in high concentration in the vacuole, may crystallize there in the form of needle-shaped crystals or sphaerocrystals (Fig. 114). These two types of pigment show histochemical characteristics closely allied to tannins (darkening with ferric salts, blackening with osmic acid). The flavins may also be cited. These are yellow pigments playing at the same time the rôle of hydrogen carriers and the rôle of Vitamin B_2. We have recently found them in great abundance in the vacuoles of a fungus *Eremothecium Ashbyii* where they crystallize in the form of needles or sphaerocrystals. The alkaloids are very wide-spread in the vacuoles of the phanerogams and may be recognized by testing with iodine-potassium iodide (Bouchardat's reaction), which precipitates the alkaloids in the vacuoles as brown granules.

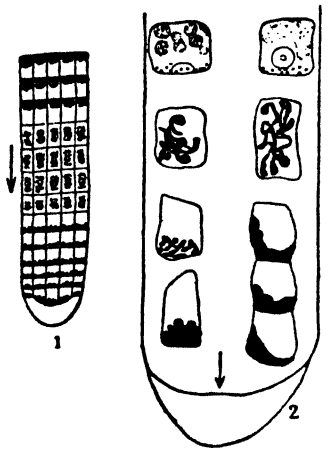


FIG. 111. — Diagrams of barley root vitally stained with neutral red and centrifuged. The chondriosome-shaped vacuoles of the meristem, heavier than cytoplasm, are displaced centrifugally; those taking in water in the region of differentiation are not affected; those still higher in the root, lighter than cytoplasm, are displaced centripetally. (After MILOVIDOV).

In bringing this inventory to a close, there must be mentioned asparagine crystals and leucine crystals (lozenge-shaped or sphaerocrystals) and especially calcium oxalate crystals. These latter are found in the vacuoles of a great number of phanerogams, sometimes as long needles (calcium oxalate monohydrate or acid calcium oxalate) belonging to the monoclinic series; sometimes in the state of octahedral crystals, isolated or twinned—quadratoctahedra, in the nature of crossed twins (calcium oxalate trihydrate)—be-

longing to the tetragonal system; sometimes as crystal dust. The crystals of calcium sulphate are also very frequently found (*Closterium, Spirogyra*).

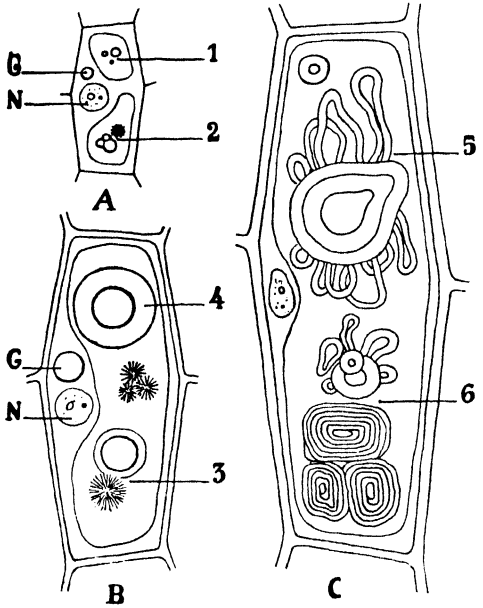


FIG. 112. — *Lilium candidum.* Lipide concretions within the vacuoles. A, in epidermal cells of the bulb; small granules (1) agglomerate into mulberry-shaped masses (2), these, B, in older scales become large bodies which are globular with a denser center (4) or of radiating crystalline structure (3). C, in drying outer scales, these break up and myelin figures (5) appear which finally, in dead cells, form masses of wound up threads (6).

Vacuolar *p*H and *r*H:- It is very difficult to obtain an exact idea of the vacuolar *p*H. It has been seen indeed that the change of color of the vital dyes has only a very questionable value. Then, too, the indicators of *p*H do not penetrate into the vacuoles and are besides very toxic. However, evaluations have been attempted by CROZIER, HAAS and IRWIN, who sought to extract the vacuolar sap of certain algae, such as *Valonia,* in which the articulations are occupied by an enormous vacuole. These evaluations have shown that the vacuolar *p*H in *Valonia* has an acid reaction (5.0-6.7). CROZIER, HAAS and SCHMIDT have used an interesting method by making the anthocyanin pigments serve as indicators. These pigments can be extracted from the petals and in mixing them with buffer solutions of known *p*H, all their different possible colors may be obtained and consequently the coloring which the petals show naturally may be related to a known *p*H. The results obtained by this method inform us as to the vacuolar *p*H. It scales from 3.0-8.0.

We have been able with GAUTHERET to find a way to evaluate the vacuolar *p*H but only qualitatively. CLARK and PERKINS have

shown that neutral red reduced in an alkaline medium (*p*H 8.2 for example) gives a leucoderivative which, by acidification of the medium (*p*H 5.2 for example), is transformed into a second derivative, yellow in color and fluorescent, which is distinct from the

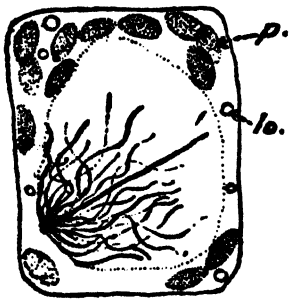


FIG. 113. — Cells of stipe of *Laminaria flexicaulis*. Bouquet crystals formed by the precipitation of iodide by cresyl blue in the vacuoles. *P*, phaeoplast. *lo*, fat globule. (After MANGENOT).

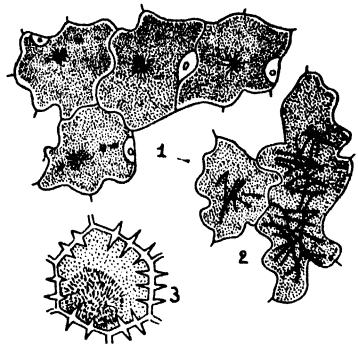


FIG. 114. — Epidermal cells of sepals. Pigment in the vacuoles, partly in solution, partly crystallized. 1-2 *Delphinium*. 1, *D. Ajacis*; pigment blue, clusters of needle-shaped crystals. 2, hort. var. with dark blue flowers; long, entangled needle-shaped crystals. 3, *Verbena* hybrid; large sphaerocrystals.

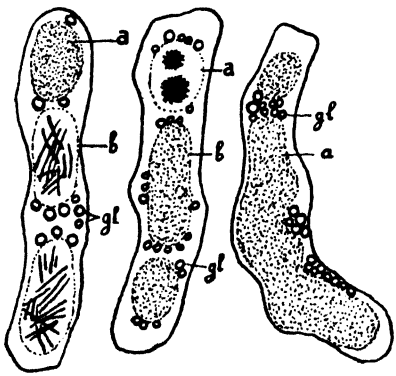


FIG. 115. — Mycelium of *Eremothecium Ashbyii*. *a*, vacuole with flavin in solution; *b*, vacuole with flavin crystals; *gl*, lipide granules.

first. This latter is also obtained when neutral red is reduced in an acid medium. Finally, although oxidation of the leucoderivative is rapid in contact with air, that of the fluorescent form is very slow. Now, in treating wheat roots, or the epidermis of *Iris* rich in phenolic compounds, with the leucoderivative of neutral red, we have found that the vacuoles take a yellow coloration which, in contact with the air, reddens only slowly. On the contrary, the cells of the bean root which are lacking in phenolic com-

pounds remain uncolored with the leucoderivative, and the leucoderivative which they have absorbed becomes oxidized instantaneously in contact with air. It may therefore be believed that the vacuoles of wheat and *Iris* are acid whereas those of the bean are not.

Vacuolar *r*H has been evaluated by means of vital stains (MATILDA BROOKS) or by micro-injection of indicators in algae such as *Spirogyra.* These measurements have given an *r*H of 16-18.

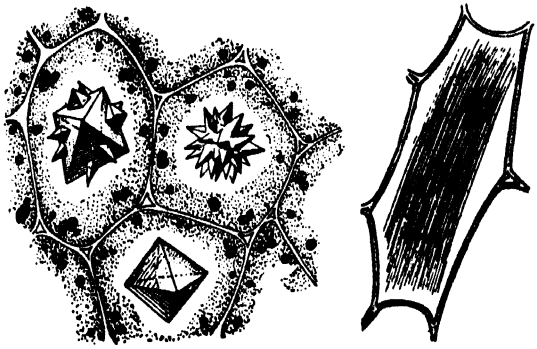


FIG. 116. — A, Petiole of *Begonia;* octahedral crystals, isolated (lower cell), or twinned (upper left), in the shape of a sea urchin (upper right). B, Leaf of *Aloë succotrina;* raphides.

ORIGIN AND SIGNIFICANCE OF THE VACUOLES

Aleurone grains: their formation. — Aleurone grains are found in every seed. Their significance has been discussed for a long time. These grains are to be found in large numbers in all cells of the embryo and are especially voluminous in the cells of the cotyledons or of the endosperm. In the Gramineae they are localized in the protein layer of the endosperm.

Aleurone grains vary in structure. In many cases (*Ricinus, Cucurbita Pepo*) they are composed of an amorphous protein mass enclosing a crystalloid of the same nature and one or several spherical bodies called globoids, formed of phytin. Sometimes twinned crystals of calcium oxalate are found in the protein mass or in the globoids. In other cases the aleurone grains do not contain crystalloids and enclose in their protein mass only numerous globoids (Gramineae). Lastly, there are cases in which the aleurone grains are composed entirely of amorphous protein (Legumes).

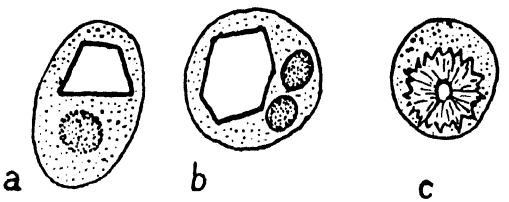


FIG. 117. — Aleurone grains in seeds. *a, b, Ricinus;* one crystalloid and 1-2 globoides. *c, Oenanthe Phellandrium;* sphaerocrystal of calcium oxalate in protein ground substance.

Two opinions have been formulated as to the origin of aleurone grains. Some authors considered them to be plastids in which were formed protein and the globoids. Others contended that they resulted from solidification of the vacuoles during dehydration of the seed. This latter opinion is now demonstrated to be true.

If, during the maturation of the seed, the vacuoles from any part of the embryo or endosperm are examined using vital stains, liquid vacuoles are found which are more or less large, according to the type of cell and its state of development. These vacuoles contain protein substances in solution and neutral red causes a precipitation of these proteins within the vacuoles as deeply stained bodies. In the course of development of the seed, the vacuoles become much richer in protein. In the stages immediately preceding maturation, *i.e.*, at the time when dehydration takes place in the seed, it is observed that the vacuoles sometimes have a tendency to break up as they lose water, becoming smaller and smaller, and less and less fluid. They stain deeply and homogeneously and are now semi-fluid. They often at this moment become filamentous or reticulate, analogous in form to those observed in meristems. Later when the seed has reached maturity and passes into the dormant stage, the semi-fluid vacuoles by more marked dehydra-

tion take on the appearance of solid, spherical, very refractive bodies. These bodies can, by crushing, be expelled from the cell and then will not take up neutral red unless they have been immersed in water for a long time.

The vacuoles are thus transformed into bodies of protein nature whose dimensions are variable. They are very small in some cells and very large in others. They are aleurone grains and are formed by the solidification of the protein contained in solution in the vacuole. As a result of losing water, a grain of protein has therefore been substituted for a vacuole. It is not astonishing, then, to

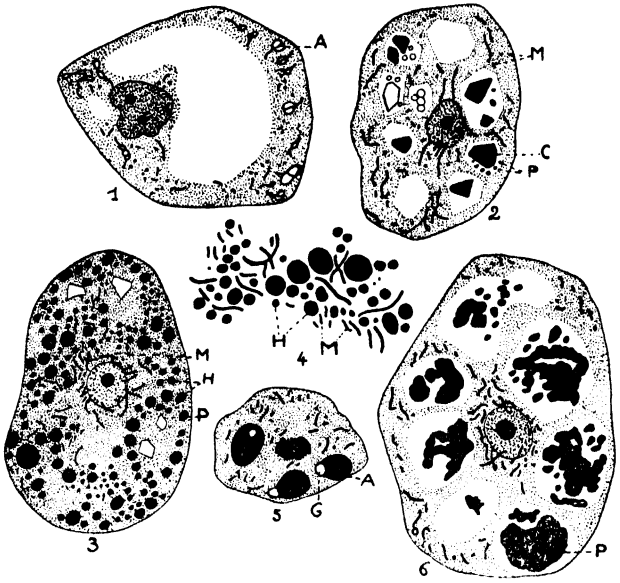


FIG. 118. — *Ricinus*. Endosperm. 1, young cell, starch (*A*) forms in chondriconts. 2, just before maturation; starch is absorbed, the large vacuole is fragmented into small vacuoles containing protein crystals (*C*) and protein precipitates (*P*). 3, oil globules (*H*) blackened by osmic acid, protein crystals (*P*). 4, detail of (3). 5, dormant seed; *A*, aleurone grain, *G*, globoid. 6, during germination of the seed; aleurone grains (*P*) beginning to dissolve. *M*, chondriosomes. 1, 2, 5, 6, Regaud's method. 3, 4, Meves' method.

find along with the protein in these vacuoles, inclusions of phytin and crystals of calcium oxalate, products encountered in many vacuoles. Some aleurone grains may even contain oxyflavanol pigments or anthocyanin. Investigations of SPEISS and CHAZE have shown that aleurone grains of some varieties of maize contain an anthocyanin pigment which gives them their characteristic black coloration. At first red, this pigment appears in the vacuoles which will later be transformed into aleurone grains, then remains absorbed by the protein of which the aleurone grain is composed. At germination, when the aleurone grains are again transformed into vacuoles, the anthocyanin pigment changes to red. Furthermore CHAZE has found oxyflavanol pigments in the yellow or white ker-

nels of other varieties of maize as well as in the seeds of other grains (barley, wheat, rye, oats). Aleurone grains stain deeply with mitochondrial techniques which do not at all stain the liquid vacuoles from which they are derived, except in periods preceding solidification when the stains bring about flocculation of the colloidal solution, and the formation in the vacuoles of precipitates stainable with iron haematoxylin.

At the time of germination, when the seed again takes up water, the aleurone grains absorb it and become semi-fluid. At this period they often show again a tendency to elongate into filaments capable of anastomosing in a network.

At the beginning of hydration, the aleurone grains stain deeply and homogeneously with vital dyes. Then the filamentous and semi-fluid, reticulate vacuoles derived from them swell as water continues to be taken in and appear as spherical liquid vacuoles which by coalescence gradually become transformed into large vacuoles in which the vital dyes cause strongly colored precipitates.

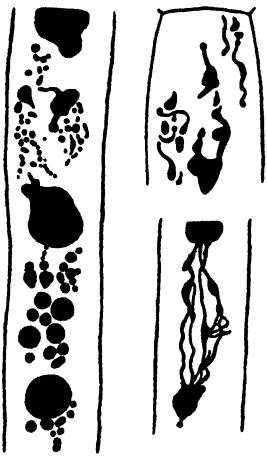


FIG. 119. — Tulip. Epidermal cells of petals of a dark red variety. Fragmentation of large vacuole, containing anthocyanin, into small, sometimes reticulate, or filamentous, vacuoles caused by plasmolysis in a 5% solution of NaCl.

In sections prepared with mitochondrial techniques, the forms which were reticulate and filamentous, at the beginning of germination, show a heavily stained, compact contour which is filamentous, or granular, and which is surrounded by a clear zone. The spherical liquid vacuoles, which succeed them, still enclose stained corpuscles but these corpuscles become less and less abundant, as more and more water is taken into the vacuoles and, finally, in the large vacuoles, no stained contents are found.

Reversibility of form in the vacuolar system. — PIERRE DANGEARD's investigations, and our own, describing this development show, therefore, that there exists a certain reversibility between the two vacuolar forms. It has previously been shown that vacuoles ordinarily appear under very different aspects according to the age of the cells:

1. As numerous, minute, semi-fluid vacuoles which have more or less the shape of the chondriosomes.

2. As a small number of large, spherical, liquid vacuoles, always containing colloidal substances, but in very dilute solutions, *i.e.*, vacuoles corresponding to the classical definition of vacuoles and capable of fusing into a single enormous element. The first of these is found in embryonic cells, the second in mature cells. If the cells are greatly dehydrated, the spherical liquid vacuoles may lose their water, become concentrated, and may again take on a semi-fluid consistency and look like chondriosomes. A more com-

plete dehydration leads finally to the transformation of these semi-fluid vacuoles into solid bodies (aleurone grains) by a solidification of their colloids. The aleurone grains, by taking up water anew, are capable of again assuming the semi-fluid consistency and appearance of chondriosomes and, by a continuance of this process, may finally become liquid vacuoles.

The filamentous appearance of the vacuoles seems to be the result of their semi-fluid state, for in the semi-fluid state, the vacuoles are generally filamentous or reticulate and stain uniformly and deeply with vital dyes. In the liquid state, the vacuoles are generally spherical and are stained only weakly with the vital dyes, which bring about a precipitation of the enclosed colloids as deeply stained granulations showing Brownian movement. The vacuoles are then composed of drops of a very dilute colloidal solution. In

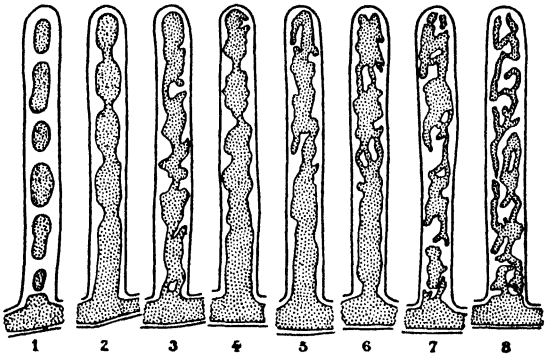


FIG. 120. — *Saprolegnia*. Modifications in form of vacuolar system in a single branch, cultivated on 1% peptone bouillon with 0.001% neutral red, in a van Tieghem and Le Monnier cell. 1-6, spherical vacuoles fuse to form a single canal which then, 7, 8, is transformed into a network (After Mlle. CASSAIGNE).

the solid state, they appear as globular bodies, which do not stain with vital dyes unless imbibition has previously taken place, but, on the contrary, always stain after fixation. The vacuoles, therefore, may pass from one form to the other depending upon the water content of the cell.

This reversibility has been obtained experimentally, furthermore, in various cells, among others, in the epidermal cells of perianth parts of red tulips (Fig. 119). In the open flower, these cells contain a large vacuole, occupying almost the entire volume of the cell, and containing a concentrated solution of red anthocyanin pigment. Now, by plasmolyzing mature epidermal cells with a strongly hypertonic solution, we have observed that these vacuoles, as they lose water, may break up into small vacuoles which become semi-fluid and appear granular, filamentous and reticulate. Similar results have been obtained in the Saprolegniaceae (GUILLIERMOND) and in the epidermal scales of *Allium Cepa* (KÜSTER).

If one observes more closely the changes in appearance of the vacuoles brought about by hydration and fusion of small vacuoles into one large one, and, inversely, if one observes the fragmentation into very numerous, small, semi-fluid, filamentous or reticulate vacuoles—a sort of splitting up of the large vacuoles—one is led to admit that in these phenomena the vacuoles play only a passive rôle. Their contraction and division are brought about by the degree of water taken into the cytoplasm which causes movements of the latter, the consequences being felt by the vacuoles. The cytoplasm, under some influences, may extract a part of the water contained in the vacuole and may swell. This swelling is therefore produced by movements of the cytoplasm, particularly by emission into the vacuoles of lamellate prolongations which finally partition off the vacuole into multiple vacuoles. These, in losing their water, become very viscous and look like chondriosomes. In the reverse process, the cytoplasm is capable of restoring to the vacuoles a part of their water of imbibition, bringing about a hydration and increase in volume of the latter. These then fuse into a single large vacuole. It is phenomena of this type which must take place at the beginning of the maturation of the seed and which must take place during the process of plasmolysis; some of the water from the vacuole passes into the cytoplasm and must cause a swelling, to which may be attributed the fragmentation of the vacuoles, and it is not until later that the cytoplasm in turn gives up its water to the exterior. In germination, the contrary phenomena must take place. The vacuole absorbs the water at first accumulated in the cytoplasm, and, as the latter continues to dehydrate, the vacuoles progressively swell and again fuse to form a very large vacuole. This view of the matter seems, moreover, to be confirmed by the fact that the viscosity of the cytoplasm increases as the plant grows old, correlative with the development of the vacuole which ends by occupying almost the entire cell.

This reversibility of vacuolar form is to be compared with a remarkable phenomenon in the tentacles of the leaves of *Drosera rotundifolia*, described long ago by CHARLES DARWIN, and designated by him as *aggregation*. While studying the modifications which occur in the pedicel of the tentacles as a result of the stimulus produced by an insect, DARWIN saw in each cell that the cytoplasm, which was colored red by pigment before stimulation, broke up before long into an aggregate of deeply stained corpuscles appearing as granules, clubs, rods or filaments showing amoeboid movements. The study of this phenomenon, taken up by various authors (GARDINER, DE VRIES, GOEBEL, and ÅKERMAN) has shown that in reality the phenomenon observed by DARWIN consists of a multiple fragmentation of the vacuole and not of the cytoplasm. The cells of the tentacle contain a single, very large vacuole filled with anthocyanin. At the moment of stimulation this vacuole undergoes a great fragmentation. It splits into a large number of small chondriosome-shaped vacuoles. Immediately after stimulation, these minute vacuoles fuse to constitute again a single large vacuole—

the cell returns to its initial state. This is therefore a phenomenon entirely comparable to that observed during the formation of aleurone grains and during the process of plasmolysis.

ÅKERMAN's work has shown that this phenomenon consists of a modification in volume of the vacuoles as a consequence of a great deal of water being taken in by the cytoplasm. The result is a fragmentation of the vacuole, caused by swelling of the cytoplasm, and at the same time an increase in osmotic pressure. This pressure increases by 5 atmospheres. Centrifuging revealed, as has been seen, that, in the stimulated cell, the vacuoles are more dense than the cytoplasm and, conversely, in the unstimulated cell, the cytoplasm is more dense than the vacuole.

The study of these phenomena has been taken up recently by DUFRÉNOY, HOMÈS, and KEDROWSKY working on *Drosera*, by QUIN-

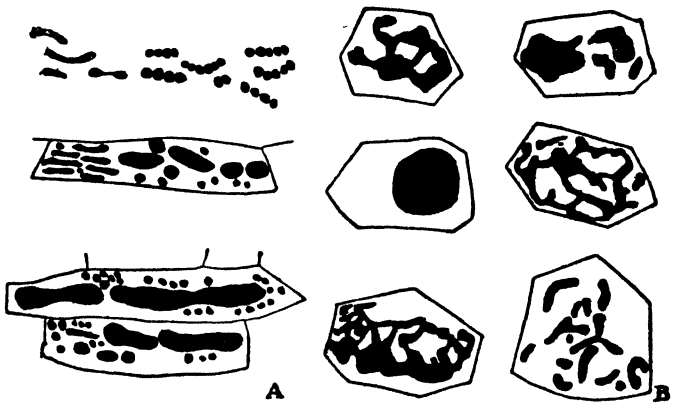


FIG. 121. — *Drosera rotundifolia.* Glandular cells in the tentacles. Vacuolar system made visible by red pigment in cells, may change from large to small, spherical, filamentous or reticulate vacuoles. A, after DE VRIES. B, after HOMÈS.

TANILHA and MANGENOT in *Drosophyllum lusitanicum.* These investigations show that it is necessary to differentiate two sorts of physiologically distinct cells in the tentacles of *Drosophyllum* and of *Drosera.* First, there are those which, covering the upper surface of the tentacles, are secretory. They excrete the complex and viscous liquid which forms a drop at the tip of each tentacle. These cells, when they are not going through a period of digestion, possess a group of small filamentous or granular vacuoles, an arrangement probably having some relation to the loss of water which takes place in the cell (QUINTANILHA, DUFRÉNOY, HOMÈS, MANGENOT, KEDROWSKY). Secondly, there are the cells which compose the lower part of the head and the pedicel. Each of these cells contains, except during the periods of digestion, a large fluid vacuole, colored red by anthocyanin. During digestion, this enormous vacuole becomes very finely divided into a large number of small, globular or filamentous elements, colored violet-grey (DUFRÉNOY, MANGENOT). These small filamentous vacuoles are oriented parallel to

the longitudinal axis, while the small vacuoles are accumulated against the proximal wall of the cell (MANGENOT).

This polarity of arrangement, which becomes more marked as digestion becomes more active, clearly indicates that these cells are traversed by a continuous flow of material caused by protein digestion (proteolesis) at the level of the extremities of the tentacles. Thus, the "aggregated" state of the vacuoles, *i.e.*, their irregular disposition, seems to correspond to differing physiological conditions—to a secretion in the cells which cover the extremity of the tentacle, to an absorption in the cells of the stalk—but both these processes indicate the passage of a current across the cells. This same arrangement of vacuoles is found in young sieve tubes in the angiosperms in which the fragmented and polarized vacuoles are very polymorphic. In these cells, large vacuoles and small filamentous or reticulate vacuoles are found. They are also found in the conducting elements of the Laminariales and the Rhodophyceae (MANGENOT) (Fig. 123).

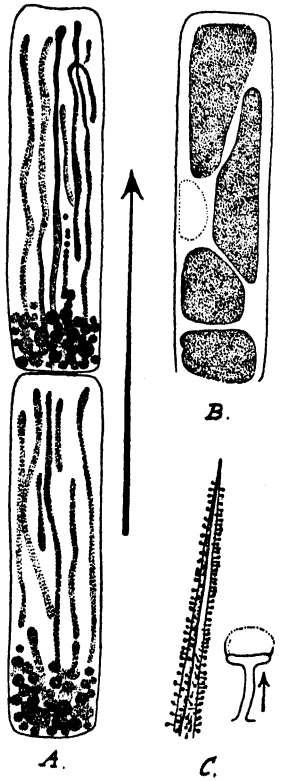


FIG. 122. — *Drosophyllum lusitanicum*. Epidermal cells of the pedicel of a tentacle. Vacuoles in grey, arrows indicate the direction of the head of the gland. A, during digestion of protein. B, during inactive period. C, tip of leaf with tentacles; one gland showing pedicel, head and drop of secretion. (After MANGENOT).

Recent work seems to suggest that the vacuoles undergo a similar fragmentation each time that the cells are in the process of active secretion (MANGENOT, Mlle. PY, THOMAS, GUILLIERMOND). The reason for this is not yet known.

Another phenomenon to consider here is that of the frequent changes in form of vacuoles in many cells. The observation of a species of *Saprolegnia* in van Tieghem and Le Monnier cells, grown in a nutrient solution to which neutral red has been added, made it possible to record this phenomenon under excellent conditions (Fig. 120). In the extremities of growing filaments the vacuoles generally appear as very small elements shaped as granules, rods or filaments. These elements are carried along by cytoplasmic currants which cause them to change shape constantly. They are capable of swelling and of contracting, of passing from the shape of granules to that of filaments and conversely. In the space of a few seconds, they are frequently seen to fuse and form rather large spherical vacuoles which, themselves, may give rise, by budding, to small vacuoles, or may be completely split up into a multitude of small elements which

then elongate and anastomose to form a network. So the transformation from large vacuoles to a network and the converse transformation are here again observed. It is only a little farther away from the tip of the filament that all the vacuoles fuse to form a vacuolar canal (Mlle. CASSAIGNE).

A prolonged observation of growing yeast without the aid of vital stains makes it possible to see that the large vacuoles, which appear rather stable, in these fungi are themselves subject to changes in shape. After a period of stability their contours may suddenly become irregular, angular, may vary continually, contracting and dilating and finally come back to a temporarily stable form. Often they are seen suddenly to put out long and thin prolongations, which later contract as the vacuole returns to its spherical shape.

Ultramicroscopic observation of the vacuoles of fungi (in cases where it is possible because of the faintly luminous outlines of these elements) has shown also that their contours often manifest slowly undulating movements. These phenomena seem to be caused here not only, (1) by differences of imbibition between the cytoplasm and the colloidal contents of the vacuoles and (2) by movements of the cytoplasm, but also by modifications of surface tension.

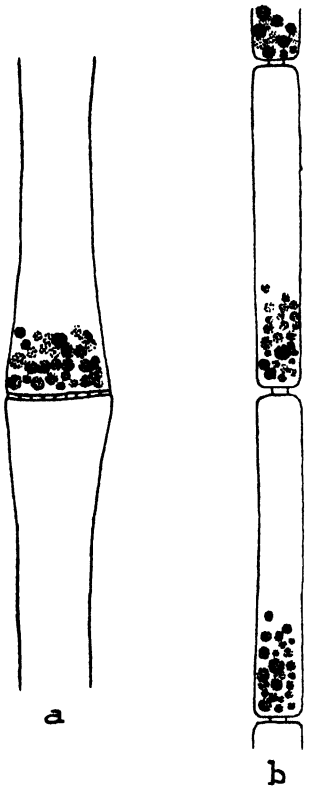


FIG. 128. — a, *Alaria esculenta* (Laminariales), b, *Bonnemaisonia asparagoides* (Rhodophyceae). Globular vacuoles at one end of cell; plasmodesmic threads connect the cells in the conducting tissue. (After MANGENOT).

The phenomenon of vacuolar contraction:- In addition to these phenomena of vacuolar fragmentation, there must be cited here another phenomenon of quite a different nature, namely, a particular state of the vacuoles which was first observed by WEBER. In the flowers of the Boraginaceae (*Symphytum tuberosum, Anchusa italica* and *Mertensia sibirica*), this investigator observed that the vacuolar sap of cells is a jelly, capable, under certain conditions, of contracting "spontaneously" *i.e.*, quite aside from all plasmolysis. This phenomenon has been observed in flowers infiltrated with solutions containing from 1%-0.1% of neutral red. There is then produced a contraction of the colloidal contents of the vacuole whose contours are curiously parallel to those of the cell itself. WEBER proposes to call this phenomenon *vacuolar contraction* and interprets it as a *syneresis*, a name given by GRAHAM to the spontaneous con-

traction of a gel when liquid is expelled. The phenomenon presupposes the existence in the vacuole, around the contracted jelly, of a liquid phase (serum) produced at the moment of contraction.

This phenomenon of vacuolar contraction seems to be very general indeed. There is frequently encountered, in the epidermal cells of flowers, a colorless space enclosing a contracted intravacuolar mass and surrounded at the periphery of the cell by a thin cytoplasmic layer. Neutral red may be superimposed upon the natural color of the vacuolar sap, when it is rich in anthocyanin compounds, and will stain the contracted vacuolar mass intensely. Careful observation of the colorless space reveals that it is not empty. It is occupied by a fluid. Plasmolysis of this modified vacuole, moreover, is quite possible and water is lost from the peripheral colorless fluid, which appears at first very poor in dissolved material. Little by little, over a period of from 24-48 hours, however, the fluid reddens. The reddening never reaches the intensity of that of the contracted mass. It is evidence, however, of a slow penetration of colloidal material into the peripheral liquid. Then a *second contraction* is frequently produced at this time. The fluid separates into a new colorless peripheral serum, and into a deeper red region, contracted about the mass originally isolated.

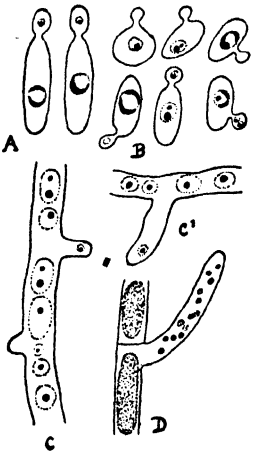


FIG. 124. — A, B, *Saccharomyces ellipsoideus* vitally stained with neutral red, vacuoles in buds seem to form *de novo*. C, D, *Oidium lactis*, vacuoles in branches seem to form *de novo*.

WEBER thinks that the same phenomenon may explain the presence in many phanerogam cells of two categories of vacuoles which are adjacent in the cell, the one liquid and lacking in tannin, the other formed of a tannin jelly. These will be taken up later. WEBER underlines also the potential importance of this phenomenon in the realization of rapid changes in the turgidity of cells, perhaps in the mechanism of certain organ movements. He points out in this regard that the motor swellings of leaves of the Leguminosae (*Mimosa*), as MANGENOT has shown, have vacuoles containing a tannin jelly, side by side with small vacuoles which do not contain colloidal substance. WEBER compared this vacuolar contraction to the natural production of large colloidal corpuscles observed in the vacuoles of the mature cells. This contraction can not be attributed to a phenomenon of syneresis, for it consists only in the partial precipitation of the vacuolar colloid. It seems, on the contrary, to correspond, as we have said, to the formation of a coacervate within the vacuolar sap.

Origin of vacuoles:- P. A. DANGEARD and then P. DANGEARD, as a result of his own work on aleurone grains, were led to adhere to the theory of DE VRIES and WENT and to admit that the vacuoles

are never formed *de novo*, but always arise by the division of pre-existing vacuoles, and are transmitted by division from cell to cell. But the DANGEARDs believe that it is not the vacuole itself, that is transmitted, but the metachromatin, which they consider to be the universal substance of their vacuome. According to them, this metachromatin persists in a solid state after the disappearance of the vacuole in the seed, as well as in the spores of fungi, and reforms vacuoles anew at germination by taking in water again. This theory is difficult to admit in view of the fact that we know that there is no single chemical substance which is characteristic of vacuoles.

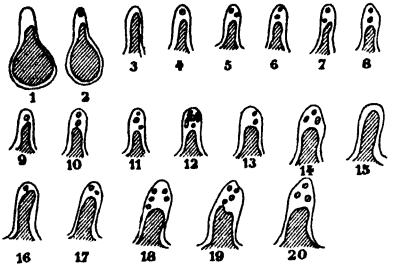
Actually, it is extremely difficult to study the origin of vacuoles in the phanerogams because of the great number, and the small size, of these elements in embryonic cells. It is known, moreover, from what has just been said that vacuoles exist in all cells and that they are capable of dividing and of fragmenting. It has been observed besides that during mitosis the vacuoles are distributed between two daughter cells. It is for this reason that BAILEY and ZIRKLE, without committing themselves on this subject, say that they have never seen vacuoles form *de novo* and that nothing proves that this phenomenon is possible. But neither the fact of the distribution of the vacuoles between the daughter cells during mitosis, nor that of their persistence in the aleurone grains of the seed and their transmission to the embryo, proves that the vacuoles can not rise *de novo*. Moreover, we can scarcely permit ourselves to consider them as individualities of the cell, incapable of forming *de novo*, when through their extreme instability of form, they may in the space of a few minutes be split up into very minute elements capable soon of fusing again.

Some fungi are more favorable for the study of the origin of the vacuoles than are the phanerogams. In the mycelium of *Penicillium glaucum* (Fig. 101) or of *Oidium lactis*, for example, lateral branches may be observed to form from filaments already containing large vacuoles and in these branches, which at first do not have them, small globular vacuoles are seen to appear which can hardly have any relation to the large vacuole of the filament from which the branch arises. This is also true of the buds of the yeasts in which there are small vacuoles which do not appear to be derived from the large vacuole of the mother cell. We concluded from these very clear facts observed by means of vital dyes that vacuoles may be formed *de novo* (Figs. 124-126).

P. DANGEARD has objected, and with reason, that vital stains can cause alterations of the vacuoles, for example, their immediate fragmentation when in the process of dividing. It is certain that vital dyes stop the multiplication of cells in certain cases, particularly in the fungi. DANGEARD again took up the study of the formation of vacuoles in yeasts and in following the budding of these fungi in a moist chamber without vital staining, showed that the large vacuole of the mother cell always puts out a delicate prolongation into the bud. The extremity of this prolongation is cut

off and forms the small vacuole of the daughter cell. DANGEARD has sought more recently to demonstrate, but this time with vital dyes, that the vacuoles of algal zoospores are always transmitted by means of the filament put out at germination.

In *Saprolegnia,* growing on media to which neutral red has been added and observed in van Tieghem and Le Monnier cells, we have shown, however, that the vacuoles which in the zoospores appear as small granules, fuse at the moment of germination to constitute a single large vacuole and then, in the germination tube, small globular vacuoles appear which do not seem to be derived from the large vacuole of the zoospore. Now, if the objection of PIERRE DANGEARD is sound in regard to vital staining of the ordinary fungi which, when carried out between slide and cover



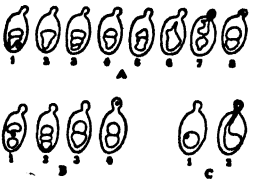


FIG. 125 (left). — *Saprolegnia.* Germination of zoospores in a van Tieghem and Le Monnier cell on 1% peptone bouillon with 0.001% neutral red. 1, 2, zoospore. 3-20, germination tube. The large vacuole extends into the germination tube and may fuse (15) with small vacuoles which form at the tip. (After Mlle. CASSAIGNE).

FIG. 126 (right). — *Saccharomyces pastorianus.* Types of formation of vacuoles during budding of the yeast grown in a van Tieghem and Le Monnier cell without vital dyes. A, 1-8, Observation during 1 hr. 1-5, successive fragmentation and fusion. 6, 7, prolongation sent into bud. 8, separation from vacuole of mother cell. B, vacuole of bud formed *de novo.* C, vacuole of bud formed by a kind of budding from vacuole of mother cell. (After Mlle. CASSAIGNE).

glass do not grow as long as they keep the neutral red accumulated in their vacuoles, this objection is evidently not sound in the case of *Saprolegnia,* which can be grown on media to which neutral red has been added. Mlle. CASSAIGNE repeated this study and observed the development of vacuoles, both in the germination tube and in the growing filaments. She was able to see small vacuoles form *de novo* which later refused to constitute a single large vacuole, or elongated into filaments and anastomosed into a network. Nevertheless, since the conditions of observation were admittedly abnormal because of the presence of neutral red, Mlle. CASSAIGNE repeated the work of P. DANGEARD on the yeasts in which she followed budding without vital staining. Now, she observed that actually the vacuole of the bud may arise from the budding of the vacuole of the mother cell, as DANGEARD indicated, but that often this vacuole also arises *de novo* in the bud.

These observations seem therefore to furnish proof that these vacuoles form *de novo.* In consequence of our research, we have

proposed an hypothesis to explain the formation of vacuoles. This hypothesis is based, on the one hand, on the fact that colloidal substances contained in the vacuoles are of very diverse constitutions, and, on the other hand, on the fact that the cytoplasm is constantly the locus of secretory phenomena (production of reserve or of waste products). The hypothesis assumes that among these products, those which are in a colloidal state separate by an unknown physical-chemical mechanism from the cytoplasm in the form of colloids non-miscible with the cytoplasmic colloids and composing a distinct phase of the latter. They appear in the form of small elements. These, by virtue of their semi-fluid consistency and of their physical state, which is rather like that of the chondriosomes, are subject to the same laws which determine the shape of the chondriosomes. This explains the resemblance in form of these two elements. According to our hypothesis, these colloids possess a capacity for taking up water which is stronger than that of the cytoplasm, and when the cytoplasm has reached its maximum point of imbibition, the excess water is absorbed within these elements which are gradually transformed into a true solution and constitute the vacuoles. In these vacuoles during the different stages of their development, there may accumulate by absorption, according to this theory, all the products secreted by the cytoplasm which are capable of forming solutions or pseudosolutions within the vacuoles. This hypothesis, which resembles that of PFEFFER, would apply at least to a great number of cases but probably not to all.

The presence in some cells of several distinct categories of vacuoles:- Recent research by MANGENOT has drawn attention to the existence of two distinct categories of vacuoles which are observed in the mature cells of numerous plants. They were glimpsed and very briefly cited some time ago by WENT, KLERCKER and LLOYD. Vacuoles which are rich in tannin, and very refractive, and which reduce osmic acid instantly, are often observed in the same cell side by side with, but in reality distinct from, other vacuoles which do not contain tannin, are very slightly refractive and show no reaction with osmic acid. The respective dimensions of each are sometimes the same, or again the tannin-containing vacuoles may be much more voluminous than the others, or conversely, may be smaller, in which case they may appear as filaments or small granules scattered in the cytoplasm around the vacuoles. Vital dyes apparently stain these two categories differently. Cresyl blue, for instance, stains the vacuoles containing tannin, blue or green, and the other vacuoles, violet or rose. Cells with tannin-containing vacuoles are very widespread in plants (Legumes, *Mimosa, Berberis, Eucalyptus, Oxalis, Monotropa, Hypopitys*).

These two categories of vacuoles, the one acid and rich in tannins, the other without tannins and seeming to have a high *p*H, BAILEY has found in the cambial cells of gymnosperms and arbores-

cent angiosperms and MILOVIDOV pointed out their existence in epidermal cells of rose leaflets.

More recent research has made it possible to show the rather frequent presence of specialized vacuoles in epidermal cells of

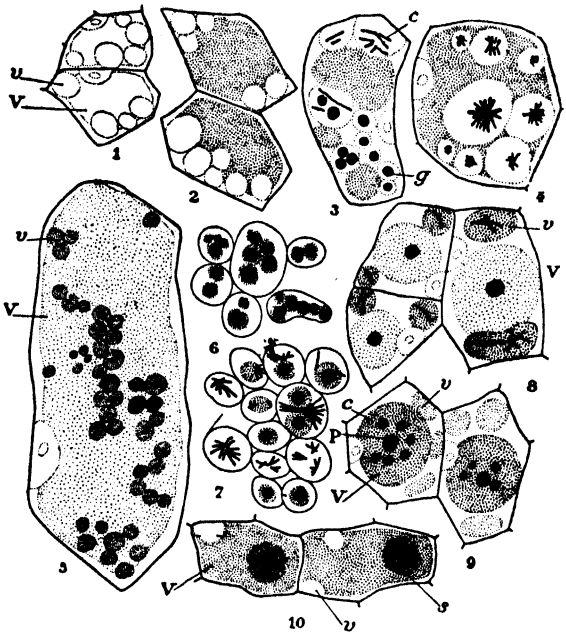


FIG. 127. — 1-7, Fruit of *Rubus fruticosus*. Two types of vacuoles. 1-4, exocarp. 1, young green fruit; large and small colorless vacuoles (V,v). 2, ripening fruit; large vacuole with raspberry-red anthocyanin pigment and small colorless vacuoles. 3, ripe fruit; three vacuoles with red pigment, numerous small vacuoles either with colloidal bodies (*g*) or needle-shaped crystals (*c*), both colored violet by anthocyanin. 4, ripe fruit; one large vacuole with red pigment, smaller vacuoles with crystals of dark violet pigment, isolated or in bundles. 5, mesocarp, ripening fruit; large vacuole (*V*), with dilute solution of raspberry-red pigment, numerous small vacuoles (*v*), with brick-red pigment and raspberry-red colloidal granules. 6, 7, mesocarp, ripe fruit; small vacuoles with one or more violet colloidal bodies, sometimes also with needle-shaped crystals of pigment, isolated or in groups, sometimes with the crystals only. 8, *Wisteria sinensis;* epidermis of petal; large central vacuole (*V*), with red-violet pigment and small peripheral vacuoles (*v*), with a concentrated solution of blue-violet pigment and crystalline needles of dark blue pigment. 9, *Hibiscus syriacus;* epidermis of petal; large central vacuole (*V*), with red pigment and dark red tannin bodies (*P*); small peripheral vacuoles (*v*), with mauve pigment. 10, *Canna indica;* epidermis of leaf; large vacuole (*V*), with red pigment and a large spherical crystal (*S*); one or more small colorless peripheral vacuoles. (*in vivo*).

leaves, fruits, and flowers which contain anthocyanin pigments. One of the most curious cases is to be found in the epidermis of the petals of *Wisteria sinensis,* already reported by WENT, in which all the cells show two very distinct categories of vacuoles: one large central vacuole and several small peripheral vacuoles. The large central vacuole contains tannin and a reddish violet antho-

cyanin pigment. The small peripheral vacuoles lack tannin and contain a very concentrated solution of bluish violet anthocyanin pigment which is capable of partially or totally crystalizing into long needle-shaped, dark blue crystals. Another no less interesting example is seen in the exocarp and mesocarp of the fruit of *Rubus fructicosus,* in which all cells likewise possess two sorts of vacuoles, the one large, solitary and centrally placed containing, at the same time, tannin and a cherry-red pigment; the other, small, spherical, extremely numerous, and scattered in the parietal layer of the cytoplasm. These latter are without tannin and form at first a brick-red pigment, but when the fruit is mature, there appear in each of these vacuoles, large colloidal bodies, dark violet in color, which show concentric zones. These are the result of the precipitation of the colloidal content of the vacuoles which has absorbed the pigment contained in the vacuoles. At maturity the vacuolar sap changes from brick-red to pale violet, then to white, whereas blackish, violet-blue crystals shaped like needles, or sphaerocrystals, are deposited in the interior of the vacuole between the colloidal bodies. In certain parts of the epidermis of the petals of *Hibiscus syriacus* also, there are found in each cell a large central vacuole, enclosing tannin as well as a raspberry-red anthocyanin pigment, and small peripheral vacuoles enclosing a mauve pigment.

In all the cases which we have just examined, the two categories of vacuoles contain colloidal substances and have the property of accumulating vital dyes, but this is not, however, universal. In a very great number of cases (in the epidermis of leaves, stem and petals of roses, in the petals of *Lathyrus odoratus, Prunus japonica, Camellia japonica, Tropaeolum majus,* in leaves of *Canna indica,* etc.), there are found together constantly, in each cell, two categories of vacuoles: a large central vacuole containing tannins or other colloidal substances as well as an anthocyanin pigment, and small colorless vacuoles seemingly without any colloidal substances. Those of the second category sometimes contain very minute crystals showing Brownian movement. In the elongated cells of the inner portion of the fleshy pericarp of the fig there are two categories of vacuoles of very curious appearance, each varying both in number and dimension in the cell. One type contains violet-red anthocyanin pigment together with colloidal substances and is very variable in shape, the larger among these having irregular contours which give them an angular appearance, the smaller ones being chondriosome-shaped elements. The other type is colorless, lacking in colloidal substance and all of them, no matter what their dimensions, appear perfectly spherical. In this case, the vacuoles which do not contain tannins or other colloidal substances never stain, which seems therefore to add further proof that vital staining of the vacuole is due exclusively to the presence in them of colloidal substances. The case of the pericarp of the fig is particularly interesting because it shows us that the shape of the vacuoles, whether irregular or like that of the chondriosomes, seems

to be attributable to the viscosity of their contents, since the coexisting vacuoles without colloidal substances are always spherical.

In all cases in which the cells contain two categories of vacuoles, these vacuoles become distinct very early and it is very difficult to determine their origin. It would seem, however, that the vacuoles lacking in colloidal substances arise by exudation from vacuoles rich in colloids, for, by plasmolysis, it is possible in some cases to obtain experimentally the formation of similar small vacuoles in cells which do not contain any.

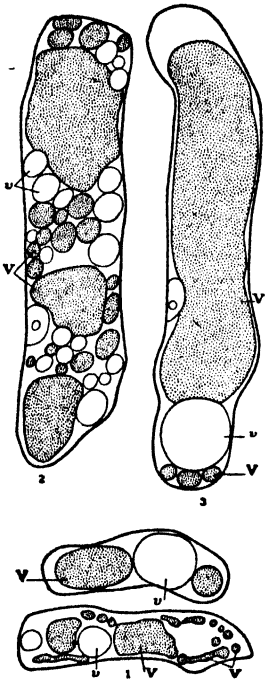


FIG. 128. — Fig. Living cells from the inner part of the fleshy receptacle. Two types of vacuoles, one (*V*) varying in size and shape with colloidal contents and anthocyanin pigment; the other (*v*), varying in size but always spherical, without colloidal substance.

It would be natural, therefore, along with WEBER and KÜSTER, to relate this phenomenon to vacuolar contraction and to attribute it to a syneresis assuming that the vacuoles not staining with vital dyes are totally lacking in colloidal substances. But we have seen that this is not always so and in the fruit of the blackberry there exist two categories of vacuoles both of which contain colloidal substances. In this case it might be supposed that these two categories of vacuoles, which seem to correspond to small accumulation and transportation centers for various metabolic products, are always distinct and have no genetical connection, or else that they arise by a differentiation from a single category of vacuoles, but by the phenomenon of coacervation and not of syneresis (*Cf.* p. 177).

However this may be, this last explanation does not apply to the lower plants, in particular to the algae, in which there are encountered still more frequently, several categories of vacuoles in a single cell. In the brown algae it has been known for a long time that there exist viscous inclusions which have been called *fucosan granules* (HANSTEEN), or *physodes* (CRATO), whose morphological significance has been the subject of numerous discussions. These inclusions stain vitally like the vacuoles and yet are present at the same time with other large vacuoles whose contours are more fluid and which also take the vital dyes but stain differently. Cresyl blue, for example, gives the inclusions a greenish blue tint, whereas the vacuoles take a violet-blue color. These inclusions contain in fact catechin tannins, showing the phloroglucinol-hydrochloric test, which explains the greenish blue color which they give to cresyl blue. Although these phenolic inclusions are always separated from the other vacuoles even in the beginning, it seems logical to consider them, as does MANGENOT,

as corresponding to specialized vacuoles, for the same reason that we consider as specialized vacuoles, those encountered in the phanerogams in which group of plants phenolic compounds are always localized in vacuoles, often having forms corresponding to those of the physodes. CHADEFAUD, who recently described similar physodes in the Phaeophyceae, thinks, on the contrary, that these inclusions are chondriosomes which elaborate mucilages and phenolic compounds. This opinion does not seem plausible to us.

The same peculiarities are found in *Vaucheria* in which the recent work of MANGENOT has shown, apart from the central vacuolar canal previously discussed, the existence of numerous small, peripheral rod- or granule-shaped vacuoles formed of a very concentrated colloidal substance. These small vacuoles which P. A. DANGEARD confused with the chondriosomes, take a blue color with cresyl blue whereas the vacuolar canal stains violet. MANGENOT thinks these small vacuoles are composed of mucilages with which, in a great many cases, tannins are associated.

In *Euglena viridis*, as well as small vacuoles composed of a concentrated solution of metachromatin, there are found in the sub-cuticular cytoplasmic layer, spheres colored purple-violet by cresyl blue and appearing as small vacuoles. According to CHADEFAUD, these are specialized vacuoles containing mucus (Fr. *corps mucifères*). In this same region in other Euglenas, there are observed elements shaped like bacteria, colored blue with cresyl blue, rarely violet, which are capable of ejecting their contents as a long filament. They seem to correspond to trichocysts such as are observed in certain ciliates.

In *Cladophora* (Fig. 103), P. A. DANGEARD cited two categories of vacuoles, those centrally placed which are large and liquid, others at the periphery which are small, semi-fluid elements shaped like rods.

The existence of specialized vacuoles (with the exception of those lacking in colloidal substances whose existence appears to be connected with a phenomenon of syneresis), shows us that it is not possible to consider the vacuome as a morphological entity in the sense of DANGEARD or to adhere to the theory of DE VRIES. It is difficult, moreover, to keep for the term "vacuole" its classical meaning and to limit it to liquid inclusions of the cell, since it is now established that the well characterized vacuoles of the majority of plants are themselves derived from semi-fluid inclusions whose consistency is often greater than that of the cytoplasm itself, vacuoles which during the development of the cells again pass through semi-fluid and even solid phases. Furthermore, we have seen that in some lower plants the small inclusions, which, by the nature of their contents and their predilection for vital stains, correspond unquestionably to the vacuoles of more evolved plants, may remain constantly in the semi-fluid state.

One fact, however, stands out very clearly from the investigations just reviewed. It is that the protoplasm itself, *i.e.*, living matter, is incapable of being stained with vital dyes except in a transi-

tory way. Either it excretes them into the vacuole or else it dies, poisoned by them. It is only in products resulting from its metabolism that the stains accumulate. We are thus brought back to the idea expressed by many cytologists, VON MÖLLENDORFF among others, that vital dyes normally stain only that which we call the deutoplasm, or paraplasm, in which are grouped all the products arising from protoplasmic elaboration. The vacuoles belong in this category and perhaps it would be suitable to include under the general heading of *vacuolar system* (a term preferable to that of vacuome, which involves the idea of morphological entity) all the paraplasmic colloidal inclusions of the cytoplasm which are not of a lipide nature or, at least, in which the lipides do not constitute the essential element. These inclusions are composed of aqueous solutions of colloidal substances elaborated by the cytoplasm, not miscible with it (doubtless forming a coacervate system separate from the cytoplasm) and are characterized by a more or less high concentration. They are however capable under certain physical conditions and in certain cells, by reason of their capacity for taking in water which is stronger than that of the cytoplasm and often unlimited, of becoming dilute and of taking on the aspect of liquid inclusions or true vacuoles. In a word, the liquid vacuole according to this interpretation may be formed each time that there is deposited in the cell a product of secretion in a colloidal state more capable of absorbing water than is the cytoplasm. So the vacuolar system expresses a physical state, an aqueous phase, separated from the cytoplasm, and containing various more or less concentrated, colloidal and crystalloid substances of paraplasm which may, according to the nature of these substances and the conditions of the cell, have a rather high viscosity and which are able to pass from the liquid to the semi-fluid or solid state.

If, in the great majority of plants, the vacuolar system appears to us as a morphological entity, it is undoubtedly because plant cells undergo a considerable hydration, and because, from the first stages of their development, the paraplasmic inclusions whose enclosed colloids take up water, are transformed into liquid vacuoles, which run together very quickly and become a single and enormous vacuole in differentiated cells. It follows logically that all the products of metabolism capable of forming solutions or pseudo-solutions with water would collect in this single vacuole. In some lower plants and in animals, on the contrary, the cells would not undergo this hydration and the paraplasmic inclusions would generally remain in the cytoplasm as concentrated colloidal solutions, making a distinction among them more easy by reason of the chemical contents characteristic of each.

According to this hypothesis it is possible to see, as does MANGENOT, a similarity between the vacuolar system and the lipide inclusions — paraplasmic formations capable occasionally of being stained in the living state because the vital stains are soluble in lipides. In these inclusions, which are formed of neutral fats and which are found in practically all cells, there accumulate all the

products of secretion of the cytoplasm capable of being dissolved in them (phytosterol, lecithins, oils, carotinoids, etc.). They may remain scattered in the form of small inclusions in the cytoplasm, or may fuse together, as in the spores of certain fungi and in the adipose cells of animals, to constitute a single enormous fatty globule occupying the entire cell.

A distinction may therefore be made in the paraplasm between *hydrophilic inclusions* (*vacuoles*) and *hydrophobic inclusions* (*lipide inclusions*).

Be that as it may, these investigations, taken all together, show that vacuoles are present in all cells, just as are the chondriosomes. Although both are present, the vacuoles cannot, in any way whatever, be considered similar to the elements of the chondriome. There is reason to think that they have no permanence, no individuality which is transmissible from generation to generation, or as PARAT says, "Only the group is significant, only the vacuome is an entity, the expression of a cellular equilibrium, the bond in metabolism, an 'aqueous phase' whose elements disappear and are replaced by others."

Digestive vacuoles:- The theory which we have just formulated in regard to the significance of the vacuoles permits us to incorporate the vacuoles of the Myxomycetes in the vacuolar system. It is known that in this group as well as in the Amoebas, there do not seem to be any digestive vacuoles which take up vital dyes but there are vacuoles which are distinguished from ordinary vacuoles by their exogenous origin. These vacuoles arise from food particles surrounded by a little water in the cytoplasmic mass. If the hypothesis which we have formulated on the origin of vacuoles be admitted, it follows that in spite of their exogenous origin, these vacuoles are in the same category as the others, contrary to the opinion of VOLKONSKY who definitely separates them under the name of *gastriole*.

There are other vacuoles, present in the flagellate algae which are pulsating vacuoles. Their significance is still unknown.

Chapter XVI

THE ROLE OF THE VACUOLAR SYSTEM AND HYPOTHESES CONCERNING IT

One of the most important functions of vacuoles is to regulate the exchange of water which takes place in the cell by osmotic phenomena. This was brought out by the classical research of DE VRIES. We have already mentioned this function (p. 125). Now we must show the applications of it made by DE VRIES.

From his experiments, this investigator thought out a method by which the osmotic pressure of a cell might be determined. This consists in placing fragments of living tissue (for example, staminate hairs of *Tradescantia,* which have been mentioned before as particularly favorable for these experiments) in solutions of a known substance such as sugar, arranged according to concentration. There may then be found a limiting concentration at which plasmolysis is just beginning, *i.e.,* in which separation of the protoplasm from the angles of the cell wall is first detected. This limiting phenomenon is considered as a criterion and it is recognized that it corresponds to an equality in osmotic pressure: the solution is therefore isotonic with respect to the vacuolar sap.

By this method DE VRIES made known one of the fundamental laws of osmosis. By a series of progressive comparisons of different substances, it is demonstrable that they are isotonic when each produces incipient plasmolysis of cells. By this biological method, DE VRIES was able to show that isotonic solutions are equimolecular, *i.e.,* equal osmotic pressures are developed by an equal number of molecules. The method is so sensitive for sugars that DE VRIES was able to determine the molecular weight for raffinose about which chemists disagreed. Electrolytes, however, are ionized in solution and each ion, acting as a molecule, increases osmotic pressure. Consequently DE VRIES had to introduce into this law a coefficient of correction (isotonic coefficient).

Osmotic pressure of the cell sap varies according to the conditions of the life of the plant: the osmotic value is 4-5 atmospheres for aerial parts of aquatic plants, 12-14 atmospheres for cells in the root of the bean, almost 100 atmospheres for the chlorophyll-bearing parenchyma of various plants.

Normally, cells are always distended by their vacuolar sap. This rigidity is called *turgidity.* The cells are entirely comparable to a blown up balloon: the internal pressure manifests itself if the membrane is pierced by a microdissecting needle and the protoplasmic and vacuolar contents escape with force just like the air of a punctured balloon. Turgidity plays a considerable rôle in the life of plants in maintaining their rigidity. When it is lacking, the plants lose their rigidity and wilt. Cells have, moreover, the means of regulating the concentration of their vacuolar sap in

such a way that it is always hypertonic with regard to the surrounding medium. As soon as the concentration of the latter increases, the cells hydrolize their reserve starch and the resulting sugar goes into the vacuolar sap whose osmotic capacity increases. This phenomenon has been given the name *anatonosis*. The presence of colloidal substances in the vacuole suggests that their rôle is not reduced merely to osmotic actions but that they intervene also in the processes of the passage of water in and out of the cell. It is, in fact, this inward and outward passage of water, intervening alternately between vacuolar colloids and cytoplasmic colloids which explains the reversibility of form of the vacuolar system discussed earlier.

The vacuoles are accumulation regions, the reservoirs of a large number of metabolic products or of reserves, and are particularly regions of excretion of toxic substances, as the action of vital dyes tends to indicate. In the vacuoles, there accumulate all the products secreted by the cell which can be dissolved in water, forming true or pseudosolutions (proteins, holosides, heterosides, tannins, flavins, oxyflavanol and anthocyanin pigments, organic acids, alkaloids, certain lipides, mucilages and so on). These various products may appear in the meristematic cells at the very beginning of development of the vacuolar system, or at any stage whatever, during the development of the system. It has been possible to demonstrate, notably by microchemical reactions, that in the seedling of tobacco, alkaloids appear in the cells of the meristem of the root, in the chondriosome-shaped vacuoles formed by the hydration of aleurone grains (CHAZE). There have been localized also in the chondriosome-shaped vacuoles of the meristematic cells, certain heterosides, such as the saponarosides (POLITIS). This is true for tannins (GUILLIERMOND, P. DANGEARD), the oxyflavanol compounds and anthocyanin pigments (GUILLIERMOND). The vacuolar system is certainly more than a locus for the accumulation of these various products. The presence of colloidal substances in the vacuoles and their predilection for vital stains lead us to suppose, as do the DANGEARDS, that the vacuoles can exercise a rôle in absorption phenomena because of these very properties of absorption, imbibition and combination which bring about the penetration of the dyes. DEVAUX believes the vacuolar system to be the site of chemical affinities of the cell and explains that the vital dyes penetrate the cell without staining the protoplasm and accumulate exclusively in the vacuoles, *i.e.*, in the non-living parts of the cell, because the chemical affinities of the living substance are masked by reciprocal saturation. So by his theory of polarized (catalytic) membranes (*Cf.* p. 121) only the non-living inclusions of the cytoplasm, such as the vacuoles, are capable of fixing the dyes, and there is localization of protoplasmic activity on the surface presented by the protoplasm and the vacuole. It has been seen that this opinion is not justified. If, actually, some dyes, like neutral red, traverse the cytoplasm without ever staining it and accumulate only in the vacuole, this is not true for other dyes which

can stain the cytoplasm in living cells. Nevertheless this staining is only transitory and the dye moves from the cytoplasm into the vacuole. It is only after the dye has been localized in the vacuoles that the cells are capable of growing and no staining other than vital staining of the vacuoles is compatible with growth. The hypothesis of DEVAUX seems to be confirmed by the works of GENEVOIS and GENAUD, who have shown that absorption of salts by cells occurs exclusively along the cellular and vacuolar membranes. It is necessary, however, to make reservations in regard to the absence of chemical affinities from the cytoplasm, since it has been seen that certain stains may, under some conditions, be retained by the cytoplasm (pp. 18, 142). It has often been supposed that the vacuolar system is not a simple center of accumulation of metabolic products but that it is at the same time the seat of phenomena of hydrolysis and of synthesis. According to KEDROWSKY and VOLKONSKY, the vacuoles are the secretion apparatus of the cell and the seat of enzymes, particularly of proteases, but this view seems to be exaggerated. There is reason to believe that in the chemical phenomena which take place in the vacuole, it is the cytoplasm which plays the active rôle, the vacuole having only a passive rôle.

Let us add that PARAT considers that in animal cells, methylene blue is always reduced in the cytoplasm and in the chondriome (rH <12), and that it is, on the contrary, re-oxidized by the vacuoles (rH <16), which does not seem to be true in plant cells. Going back to the hypothesis of ROBERTSON (p. 122), PARAT thinks that the pair: chondriome plus vacuole, presides over the synthesis of proteins, which, according to ROBERTSON, calls for a lipide phase and an aqueous phase and thus gives a morphological basis for this hypothesis. PARAT considers further that the vacuome is the crucible in which are completed the operations begun in the chondriome, but these points of view are very hypothetical and lack a solid foundation.

It has been seen that this hypothesis of PARAT is no longer tenable, now that it is demonstrated that the chondriosomes do not of themselves have a reducing rôle, contrary to what had been supposed, and that the vacuoles may in certain cases be just as capable of reducing actions as the chondriosomes.

Chapter XVII

GOLGI APPARATUS, CANALICULI OF HOLMGREN AND OTHER CYTOPLASMIC FORMATIONS

Golgi apparatus and the canaliculi of Holmgren in animal cells:- By using methods of silver nitrate impregnation, GOLGI (1898) brought out in the cytoplasm of nerve cells (Purkinje cells and invertebrate ganglia of *Strix flammea*) a network of very fine filaments to which has been given the name *internal reticular apparatus of Golgi.* This formation was made the object of important studies by CAJAL. KOPSCH showed later that the Golgi apparatus can also be brought out by osmic impregnation at 40°C.

This later method has the advantage over the preceding that it is much easier to use, for, unlike impregnation with silver, it does not result in so many failures. For this reason, it has been the starting point for a great deal of research which has revealed in most animal cells, formations which osmic acid blackens, just as it does the Golgi apparatus described by GOLGI and CAJAL. These formations, in spite of their widely differing morphological aspects, have been grouped with the Golgi apparatus on the single basis that they stain like it. These formations are not generally represented by a network but by small elements scattered in the cytoplasm, appearing as spherical or ovoid bodies, composed of a chromophobic substance surrounded by a chromophilic substance which is thicker on one side than on the other. They are known as *dictyosomes* or *Golgi bodies.*

Many cytologists today think that the Golgi apparatus is a permanent feature of cytoplasm in the same way as is the chondriome, and there has been described, during mitosis, a division of the Golgi bodies between the daughter cells which has been called *dictyokinesis* (PERRONCITO). Finally, scientists are coming to the belief that this apparatus is the center of elaboration of metabolic products. In brief, it is thought to play the rôle formerly attributed, first, to the ergastoplasm and, later, to the chondriome. So the Golgi apparatus may be said to have supplanted the chondriome for those who adhere to this view.

The Golgi apparatus, however, is not, like the chondriome, a well defined system. It is not visible in living material nor can it be revealed by microdissection (KITE and CHAMBERS). It can be demonstrated only by methods which, as we shall see, are in no wise specific. Morphologically, it is so imperfectly characterized that BOWEN said "The Golgi apparatus is above all a substance, a cellular apparatus, whose modelling has only a secondary interest." Such a definition could only be acceptable if the Golgi apparatus were composed of a well-defined substance. Now its chemical nature is completely unknown and it does not even have definite characteristics of fixation and staining. It is not certain,

moreover, that the images obtained by osmic acid correspond always to those produced by silver methods and there has been no proof whatever that the dictyosomes can really be homologized with the Golgi network. One can not, therefore, suppress the thought that under this name have been grouped very diverse formations. One is forced to admit that there is reason to distin-

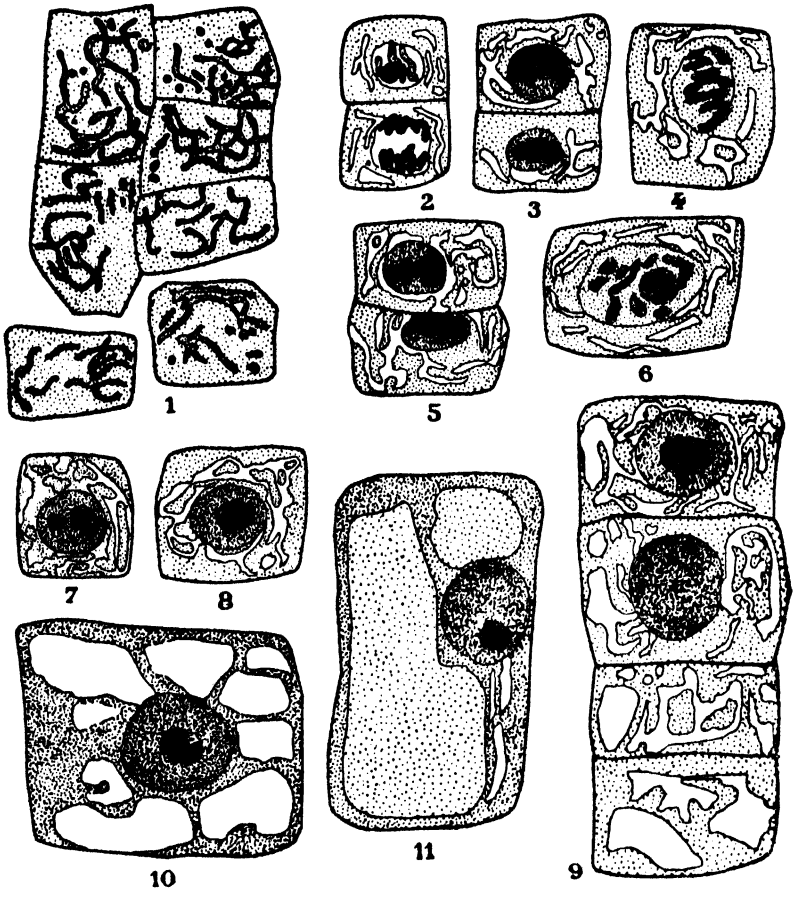


FIG. 129. — Barley root. 1, meristem vitally stained with neutral red; vacuolar system deeply stained, more or less reticulate. 2-11 Bensley's method; 2-9, meristem; vacuolar system has the appearance of Holmgren's apparatus. 10, 11, differentiating cells; Holmgren's apparatus transformed into large vacuoles.

guish between formations which are perhaps entirely different: the Golgi network of GOLGI and CAJAL obtained by means of silver methods and the dictyosomes later brought out by osmic methods.

HOLMGREN, on the other hand, by the use of special methods, described in certain animal cells a network of hyalin and colorless canaliculi appearing as clearly as if punched out of the dense and stained cytoplasm. This apparatus, called the *canaliculi of Holmgren* or *fluid canaliculi* or *trophospongium*, has been found in a great number of animal cells. HOLMGREN at first considered it as

a system of intracellular canaliculi opening freely to the exterior and serving for the entrance of nutritive juices as well as for the excretion of metabolic products from the cell. Nevertheless, after further research, this worker was led to deny all communication of these canaliculi with any part of the pericellular space and considered them to be formations completely separated from the lymphatic circulation and probably comparable to the network of Golgi. It is certain, however, that, of the formations described by HOLMGREN, some correspond to canaliculi communicating with the exterior as this investigator at first thought. But in these pages we reserve this term exclusively for the formations described by CAJAL under the name which has been currently used since then

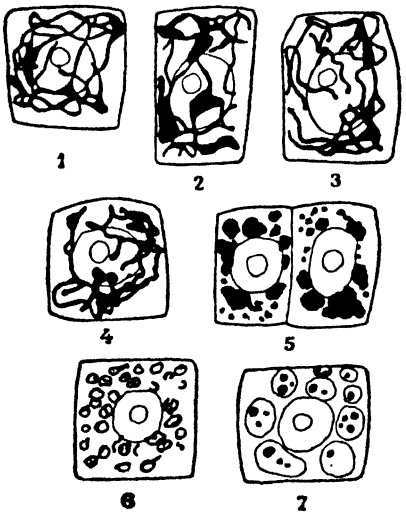


FIG. 180. — Pea root. Vacuolar system. Method of da Fano. 1-4, network strongly impregnated with silver. 5, fusion to uniformly stained vacuoles, which later (6) appear like dictyosomes, then (7) become larger with silver-impregnated precipitates.

by numerous cytologists, viz. *Golgi-Holmgren apparatus*. These canaliculi do not seem to us to be comparable to the structures now called dictyosomes.

Possible relationships of the vacuolar system with the apparatus of Golgi and of Holmgren:- The facts concerning the vacuolar system in plant cells have given the question a new orientation.

Well before the origin of vacuoles and their property of accumulating vital dyes were known, BENSLEY (1910) had succeeded in bringing out the canaliculi of Holmgren in the cells of the meristem of the root of *Allium Cepa* and had proved that they are transformed into vacuoles in the course of cellular differentiation.

Having been struck by the resemblance of the young filamentous and reticular vacuoles in embryonic cells to the formations known as the Golgi apparatus in animal cells, we had formulated, in our

very earliest investigations, the hypothesis that the Golgi apparatus might well correspond to a vacuolar system analogous to that of plant cells. Moreover, we had shown that, by means of Regaud's method, the young filamentous and reticulate vacuoles may be seen as a network of colorless canaliculi within the grey-tinted cytoplasm and present absolutely the aspect of the canaliculi of Holmgren. That led us to think that the apparatus of Golgi and that of Holmgren might perhaps be one and the same formation, corresponding to certain phases of the vacuolar system analogous to that of plant cells.

A little later, with MANGENOT, we tried to verify this hypothesis in the meristem cells of the barley root (Fig. 129) which, as we have seen, contain small filamentous vacuoles, very characteristic and easy to bring out by vital staining with neutral red. Treating these cells by the method recommended by BENSLEY for detection of the canaliculi of Holmgren, we succeeded in obtaining images very comparable to those of an apparatus of Holmgren formed of colorless canaliculi, appearing as if punched out against the grey cytoplasmic background. These, in differentiating cells, swell and coalesce and, in the mature cell, are transformed into large vacuoles. Moreover, in treating the same root with the silver impregnation methods which GOLGI employed to bring out his reticular apparatus, we obtained in the cells of the meristem a network like that of Golgi, corresponding exactly to the apparatus of Holmgren, obtained by the methods of Bensley, and to the filamentous and reticulate phases of the vacuolar system, as they appear after vital staining with neutral red.

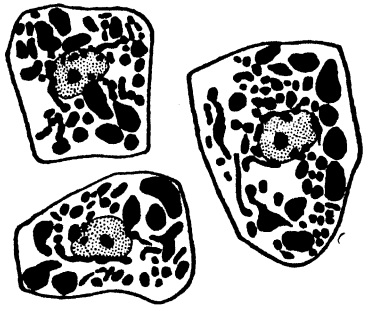


FIG. 131. — Pea. Epidermal cells of the cotyledons at the beginning of germination. da Fano's method. Aleurone grains strongly impregnated; some appear filamentous.

These observations which seemed to verify our hypothesis, were later confirmed in animal cytology by the work of A. CORTI, then of PARAT and of his collaborators. CORTI proved, in fact, that the apparatus of Golgi and the apparatus of Holmgren constitute a single formation, corresponding to a system of lacunae, which the author called *lacuome* and which he compares to the vacuolar system of plant cells. Furthermore, in entirely independent research, PARAT and his collaborators showed that the Golgi apparatus and that of Holmgren correspond to a single formation—positive and negative images, respectively, obtained by different methods and comparable to a vacuolar system like that in plant cells and capable of being stained vitally by neutral red. The research of PARAT and his collaborators have, however, proved that many of the formations assigned to the Golgi apparatus are images of the somewhat altered chondriome, or are a superposed chondriome and vacuolar system, or else are differentiated chondriosomes.

As a result of these investigations, we extended our research to cover a large number of plants, belonging to the most varied groups, which confirmed and completed our earlier findings. The study of the vacuolar system, notably in the seedling of the pea, gave us particularly suggestive results. In the meristem cells of the root (Fig. 130), there is obtained by silver methods an entirely characteristic reticulate apparatus and it is observed that during

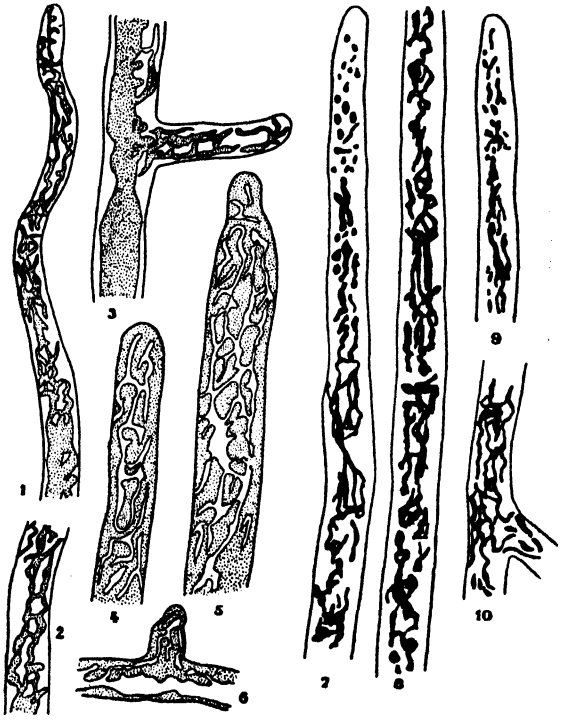


FIG. 132. — *Saprolegnia*. Vacuolar system. 1-3, vitally stained with neutral red; reticulate, tending to fuse into a vacuolar canal. 4-6, Bensley's method; system appears as canaliculi of Holmgren. 7, 8, da Fano's silver impregnation method; system impregnated with silver, resembles Golgi's network. 9, 10, Kolatchev's osmic impregnation method; system strongly blackened.

cellular differentiation this network swells and is transformed into small spherical vacuoles, each containing a precipitate heavily blackened by the silver and often arranged as a crescent on the border of the vacuoles, thus appearing like the Golgi elements or dictyosomes. Finally, in differentiated regions, the vacuoles are seen to swell and run together to form large vacuoles containing a more or less large number of corpuscles, blackened by a deposit of metallic silver. There are, therefore, obtained with silver methods, images which can be perfectly superimposed on those furnished by vital staining with neutral red.

By means of these methods of impregnation, metallic silver is deposited on the filamentous and reticulate elements of the vacu-

olar system, which gives them a homogeneous black coloration, and in the vacuoles derived by hydration of these elements, the same methods bring about the precipitation of the colloidal contents in the form of corpuscles on which the metallic silver is deposited.

The silver methods also permitted us to bring out aleurone grains during their transformation into vacuoles which swell, at first assuming filamentous forms having a tendency to anastomose,

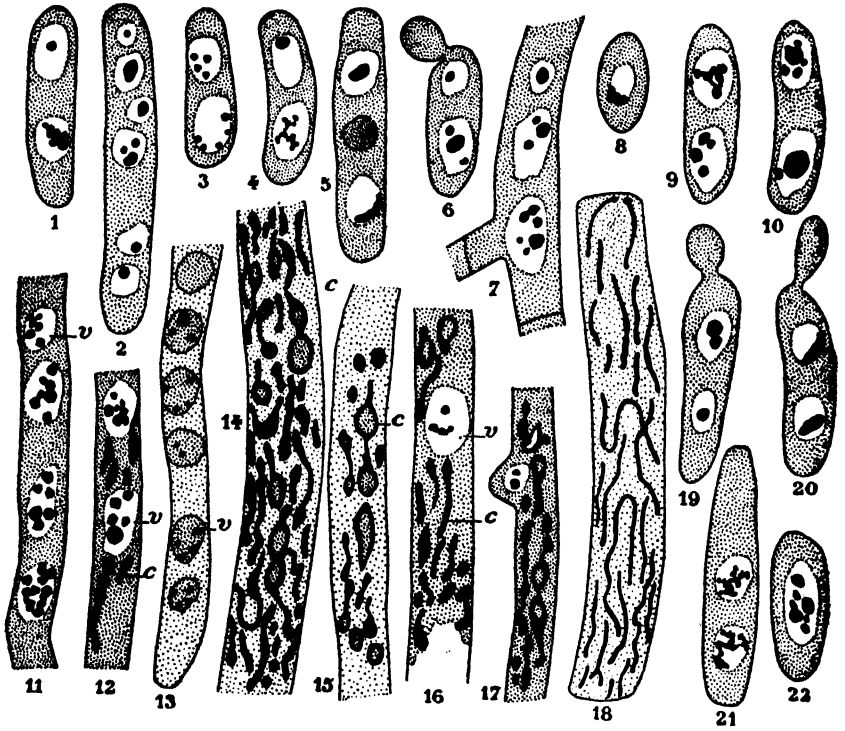


FIG. 133. — 1-6, *Saccharomyces ellipsoideus*. 7, *Ashbya gossypii*. 8-10, *Saccharomyces pastorianus*. 1-10, da Fano's method; Metachromatin bodies, produced by flocculation from the colloidal solution in the vacuole, strongly blackened by a deposit of metallic silver on their surfaces. 11-18, *Geotrichum lactis;* capricious impregnation with Kolatchev's method. 11, 13, metachromatin bodies within vacuole. 12, 16, 17, metachromatin in vacuoles (*v*); chondriosomes (*c*) are swollen and vesiculated. 14, 15, chondriosomes are swollen and vesiculated. 18, chondriosomes are well preserved. 19-22, *Saccharomyces pastorianus;* Kolatchev's method; metachromatin bodies blackened by osmium.

then later appearing as large spherical vacuoles containing numerous corpuscles which take up the silver.

Bensley's method, applied to the meristem cells of the root, brings out canaliculi entirely reminiscent of those of Holmgren. In differentiated regions of the root, these are gradually transformed into large vacuoles.

Results just as diagrammatic were obtained in *Saprolegnia*. In the extremities of the filaments, Bensley's method produced reticulate figures of the vacuolar system in the form of the apparatus of Holmgren and silver methods gave them the appearance characteristic of the reticulate apparatus of Golgi. Farther away

from the tip, they become transformed into a vacuolar canal containing numerous corpuscles which take up the silver (Fig. 132).

Silver methods gave us similar results in other fungi (*Endomyces Magnusii*, yeasts) whose vacuoles are not filamentous but begin as small spherical elements filled with metachromatin. The silver methods make these elements appear as small vacuoles, each containing one silver-staining body, whereas in the larger vacuoles, arising from the coalescence of the smaller elements, these methods bring about the precipitation of numerous silver-staining corpuscles which correspond to metachromatic corpuscles. The images obtained are here again similar at all points to those

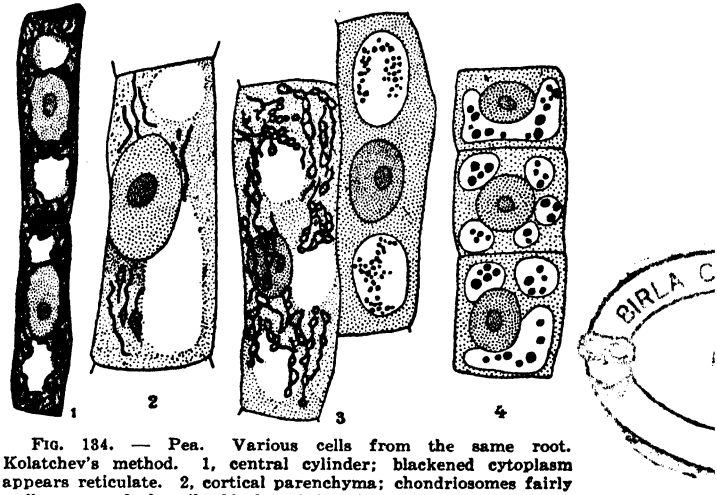


FIG. 134. — Pea. Various cells from the same root. Kolatchev's method. 1, central cylinder; blackened cytoplasm appears reticulate. 2, cortical parenchyma; chondriosomes fairly well preserved, heavily blackened by the osmium, a few are vesiculate. 3, adjacent cells; at left, only the chondriosomes and plastids are blackened and strongly vesiculated; at right, only vacuolar precipitates are impregnated. 4, Differentiated cells above the meristem; only vacuolar precipitates are blackened.

produced by neutral red. Silver impregnations also bring out the metachromatic corpuscles of some algae (*Tribonema*) and bacteria.

Research carried out by means of silver methods, regularly controlled by vital observation in cells very favorable for this, establish the fact, therefore, that the vacuoles, whatever their contents, have the property of reducing silver nitrate and of bringing about in their interior the production of particles of metallic silver, giving images analogous to those produced by vital dyes. On the other hand, silver methods, although bringing out the vacuoles very clearly, are not specific for them and sometimes the chondriome (chondriosomes and plastids) may be impregnated also and even the chromosomes (giving in that case superb mitotic figures). There is no alteration of the chondriosomes and plastids, however, so that it is not difficult to recognize them when they are blackened by the silver.

Relationships between the Golgi apparatus and the chondriosomes and plastids:- The above results have been a subject of much debate in animal cytology, and various authors, among others BOWEN and GATENBY, DUBOSCQ and GRASSÉ have not been able to confirm the observations of A. CORTI and PARAT. It must be noticed that all these authors abandoned silver methods and used only osmic methods. There was therefore the question as to whether osmic methods produce the same results as the silver methods. Work which we have done using these methods on plant cells has shown us that the osmic methods are much less specific than the silver methods. If the impregnations have lasted only a week, there is a blackening of the vacuolar system only when it encloses

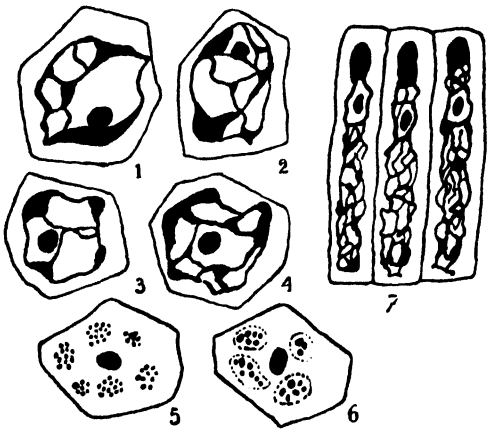


FIG. 135. — *Vicia Faba.* Cells in the seed before maturation. Silver impregnation of da Fano. 1-6, parenchymatous cells of the cotyledon; 1-4, Golgi network. 5-6, deprived of oxygen, the Golgi network breaks up into vacuoles containing silver-impregnated precipitates. 7, epidermal cells of the integument of the seed; Golgi network. (After SANCHEZ).

tannins, which instantaneously reduce osmic acid. Otherwise, it is the chondriome which is impregnated and it may be well preserved but is often vesiculated. If the impregnation is prolonged to two weeks, there is a more profound alteration of the plastids and chondriosomes, which become large vesicles and sometimes even anastomose into a fine network, much like the network of Golgi, but now the vacuolar system may also be impregnated. These impregnations are then very irregular and it is not rare to observe, side by side in the same section, cells in which the chondriome alone is blackened, sometimes well-preserved, sometimes strongly vesiculated, and other cells in which the chondriome and the vacuolar system are both blackened, still other cells in which only the vacuolar system is affected (Fig. 133, 11-18). These results demonstrate therefore that osmic methods constitute a dangerous technique, a constant source of gravest error.

Without having the pretension of entering here into a field which is not our own, we will confine ourselves to saying that

what we have observed in plant cells, under as accurate conditions as possible, leads us to think that the so-called Golgi formations observed in animal cytology have not been well characterized. Indeed, they have been observed most often by the use of methods far from specific without recourse to other techniques and without confirmation from living material. Our research on plant cells would seem to indicate that the Golgi apparatus (apparatus of Golgi, Holmgren, Cajal) *i.e.*, the network, might often correspond to a vacuolar system like that in plant cells, whereas most of the dictyosomes obtained by osmic methods must be put into the category of vesiculated chondriosomes. (An opinion recently formulated by FILHOL is that some dictyosomes correspond to differentiated chondriosomes, doubtless destined to play a rôle in the secretions of the cell). In reading the reports of some cytologists, one has the impression that they are re-discovering the chondriome under the name of Golgi apparatus.

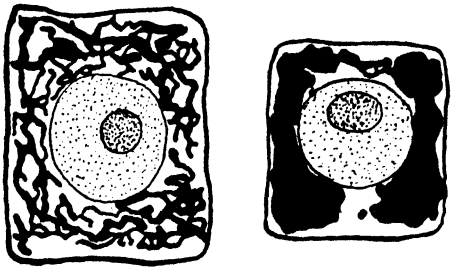


FIG. 136. — *Vicia Faba*. Golgi apparatus (vacuolar system) in meristem cells of the root. Osmic impregnation. (After Miss SCOTT).

The so-called Golgi apparatus in plant cells:- It is clearly demonstrated, at any rate, that the Golgi apparatus does not exist in plant cells. If we pass in review the various work on cells carried out with the idea of finding a Golgi apparatus, we see that all that has been described as such corresponds either to the vacuolar system or to the chondriome (chondriosomes and plastids). Thus SANCHEZ, LUELMO, GONÇALVES DA CUNHA, ZIRKLE by silver methods and Miss F. M. SCOTT by osmic methods, obtained superb Golgi networks which correspond to the vacuolar system in its filamentous and reticulate phases and which, moreover, are considered as such by these authors.

DREW, on the contrary, figured in the root of *Allium Cepa* under the name of Golgi apparatus, elements obtained by silver methods which it is easy to classify with the chondriome (chondriosomes and plastids) and which correspond exactly to that which is obtained by mitochondrial methods. On the other hand, BOWEN, then BRONTË GATENBY and his collaborators, described in a variety of plant cells, treated with osmic methods, certain elements in the form of rings. These they consider to be distinct both from the chondriosomes and from the vacuoles. These authors give to these formations the name of *osmiophilic platelets* (Fig. 138) and consider them to be Golgi apparatus. BEAMS and KING claimed that they had demonstrated the existence of the osmiophilic platelets of BOWEN and GATENBY by the following process: they subject the tip of the root of *Vicia Faba* to ultra-centrifuging by means of the apparatus of Beams, then, immediately after this process, they im-

pregnate it with osmium. After this treatment, the cells show at one of the poles corresponding to the direction of centrifugal force, an accumulation of chondriosomes and starch-bearing plastids. At the opposite pole are accumulated in order, the lipides, the vacuolar sap and the osmiophilic platelets which consequently seem to be lighter. But the figures given by the authors are not very convincing and it seems that the substance which was affected by the centrifugal force corresponds only to starch-containing plastids and that the so-called osmiophilic platelets represent vesiculated chondriosomes.

It has been seen that with osmic methods KIYOHARA obtained analogous figures (vesicles) which he interprets as normal forms of the plastids. This author, starting with a false premise in the form of a defective observation of living material in which he saw only vesiculated chondriosomes, concluded that mitochondrial methods alter the chondriosomes, whereas the Golgi methods preserve them in their vesicular form (*Cf.* p. 91). Our work, however, has furnished proof that these so-called osmiophilic platelets are none other than vesiculated chondriosomes and plastids. Finally, WEIER, not being able to find in plants a Golgi apparatus independent of formations already known, and having succeeded, in *Polytrichum commune*, in impregnating large plastids by Golgi methods, came to the conclusion that the Golgi apparatus is represented in plant cells by the plastids (*Cf.* pp. 91, 92). This opinion is however, inadmissible, for the reason that the plastids are a variety of chondriosomal elements, belonging to chlorophyll-containing cells, and in direct relation with photosynthesis which characterizes these cells, and they are not found in fungi. Besides, the ordinary chondriosomes are impregnated by Golgi methods just as well as the plastids.

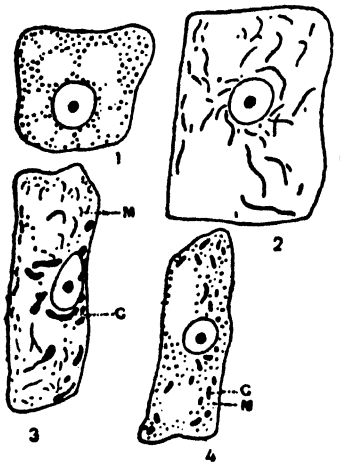


FIG. 137. — *Allium Cepa.* DREW's figures of the Golgi apparatus in the root. 1, 2, meristem; only chondriosomes differentiated. 3, 4, differentiating cells with Golgi apparatus (*G*) and chondriosomes (*M*). These Golgi elements are in reality plastids.

It must be added that GICKLHORN, studying large spherical bodies which are found localized in the vacuoles of epidermal cells of *Iris*, noticed that under the influence of osmic acid they blacken and become a spongy structure, then are transformed into a network which looks like Golgi material. This worker thinks, therefore, that it is to structures of this nature that the Golgi formations must be attributed. The work of one of our students, REILHES seems to have demonstrated that these bodies are composed of a phytosterol. LIEBALDT has recently supported this same opinion. It is possible that analogous formations have been described in

animal cells as the Golgi apparatus, but in exceptional cases only. Therefore the opinion of GICKLHORN is not a solid basis for generalization.

It is therefore demonstrated that all the formations described as Golgi apparatus in plant cells are dissimilar elements, belonging either to the vacuolar system or to the chondriome (chondriosomes and plastids) and that consequently there is no Golgi apparatus in plants.

Other cytoplasmic formations:- In the cytoplasm of many cells and especially in that of the Protista, granules of chromatin have been reported which were supposed to have originated as emissions from the nuclei. For several years, great importance was attached to these granules called *chromidia.* In reality the chromidia, as a group, have never been characterized histochemically. It has been proved, on the contrary, that they represent dissimilar elements which can be stained with iron haematoxylin and which correspond, either to chondriosomes altered by the fixatives, or to vacuolar precipitates.

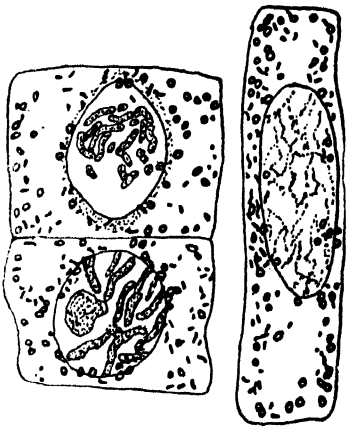


FIG. 138. — *Vicia Faba.* Osmiophilic platelets in meristem cells of the root. Weigl's method of osmic impregnation. (After BOWEN).

There is reason, also, to mention here the formations described for the first time in animal cells by the BOUIN brothers, and by GARNIER, then by PRENANT, as ergastoplasm and later found as well, in some plant cells. These are in reality rather undefined formations and appear as superposed lamellae, or as spiral filaments, which have a strong affinity for nuclear stains. They have been observed in glandular cells and an important rôle in secretory phenomena has been attributed to them. All cytologists are in agreement today in recognizing that ergastoplasmic formations have no separate existence. They are most often simply artifacts — alteration figures of the chondriosomes, plastids, vacuolar colloids, or paraplasmic inclusions, produced by fixatives. Perhaps they correspond also to the differences in chemical composition of certain regions of the cytoplasm.

Chapter XVIII

LIPIDE GRANULES, MICROSOMES AND OTHER METABOLIC PRODUCTS

Observation of living material in most, if not all, plants shows that there exist in the cytoplasm in addition to the elements discussed above, certain small granules, spherical in shape, which we call *lipide granules.* These granules have often been confused with mitochondria. In living cells observed in direct illumination, they are the most clearly visible of all the cytoplasmic inclusions because of their high refractivity. They are still more distinct with lateral illumination, under which circumstances they are usually the only visible elements of the cytoplasm. They appear strongly lighted against the black background (the cytoplasm) on which they can very distinctly be seen to move. With the Zeiss micropolychromar they can very clearly be seen to have a color different from that of the cytoplasm and much more accentuated than that of the chondriosomes. The lipide granules are distinguished very sharply from the mitochondria by their high refractivity, by their very rapid displacements in the cytoplasmic currents and by the variability of their size. The smallest have a size inferior to that of mitochondria and the largest may greatly exceed it. These granules are distinguished from the mitochondria also by their osmium-reducing properties.

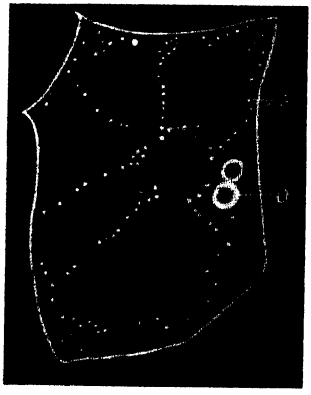


FIG. 139. — Tulip. Epidermal cell of leaf under the ultramicroscope. Only the lipide granules (*G*) carried about in the cytoplasmic trabeculae are visible. *O*, fatty body.

In some cases they may agglomerate in mulberry-shaped masses or in little chains and fuse to become huge globules. It seems that most of the bodies described as *oleoplasts* or *elaioplasts* correspond to agglomerations of granules of this nature formed under influences as yet unknown. In each epidermal cell of the leaf of *Vanilla planifolia,* WAKKER first called attention to a voluminous body which he called an *elaioplast.* It is generally larger than the nucleus and is localized in the cytoplasm and in the neighborhood of the nucleus. This body is irregular in shape and composed of very numerous small lipide droplets which according to WAKKER are enclosed in a protein film. These bodies, which were thought to be plastids elaborating lipides, have also been found in the epidermis and other tissues of many plants, especially of the Monocotyledons (Fig. 142). They cannot however be considered as plastids. In the epidermis of tulip, each cell encloses a large fatty body which

arises from the fusion of numerous small globules and appears to correspond to the elaioplasts of WAKKER. On the other hand, the bodies comparable to the elaioplasts which are encountered in the hepatics have a constitution much more complex. They are much more difficult to interpret and are still little known.

The quantity of lipide granules varies a great deal from one cell to another, according to the state of cellular development. There are cells in which they are very rare but usually they are very numerous. These granules give lipide reactions. As well as reducing osmic acid (Fig. 141), they stain with Sudan, scarlet R, tincture of Alkanna and indophenol blue. They seem to have a variable chemical constitution and the microchemical characteristics either of simple lipides or those of compound lipides, according to

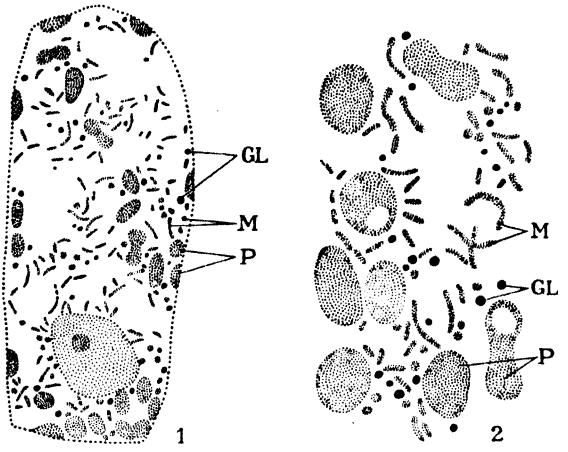


FIG. 140. — *Elodea canadensis*. 1, Cell from the leaf fixed with Meves' method, stained with acid fuchsin, which colors the chloroplasts (*P*) and chondriosomes (*M*) red; lipide granules (*GL*) colored dark brown by the osmic acid. 2, detail; some chloroplasts are dividing.

the type examined. In paraffin sections, Regaud's method does not preserve them and the osmic acid used in the method of Meves turns them brown. Sometimes they stain in frozen sections with Dietrich's and Regaud's methods. (Fig. 143).

These granules, which correspond to the microsomes of other writers, represent simple products of metabolism and perhaps in many cases they are also the product of a transitory, or final breaking down, of the lipides from the lipo-protein compounds (phenomenon of lipophanerosis). FAMIN has recently shown that their quantity increases, especially when the cells are submitted to high temperatures.

These granules attracted the attention of P. A. DANGEARD, who in his observations of living plant cells described them successively as *microsomes, spherosomes, cytosomes* and *liposomes*. This investigator made of them a permanent system of the cell which he designated first as *spherome*, then as *cytome* and then

as *ergastome*. The terms *cytosomes* and *cytome* were first created by P. A. DANGEARD to replace those of *spherosomes* and *spherome*, which were applied to the lipide granules in question here. This writer, perceiving later that, as well as these granules, there also exist chondriosomes, whose reality up to then he had refused to admit, substituted the above terms for those of chondriosomes and chondriome and made the distinction, from that time on, between the *cytome* corresponding to the chondriome and the *ergastome* which includes all the lipide granules of a cell and for which he reserved the term *liposome*. Here is the description which he gives of them (1919): "The spherome is composed of all the microsomes. The microsomes are small, very refractive spherosomes of a fatty appearance which blacken more or less with osmic acid." P. A. DANGEARD, contrary to our judgment, maintained that it is the microsomes, with the elements of the vacuome, which represent what the cytologists had for a long time been calling chondriosomes. At the present time, DANGEARD seems to have renounced this opinion. KOZLOWSKI also confused these same granules with the chondriosomes. After observing only living material, he maintained that plastids arise by simple agglomeration of these granules.

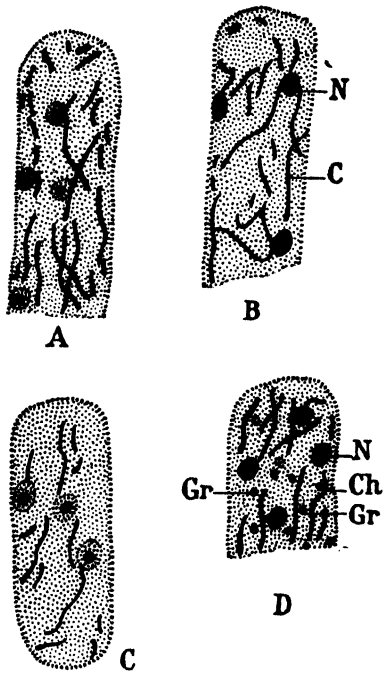


FIG. 141. — *Endomyces Magnusii*. A-C, Regaud's method; D, Meves' method; stained with haematoxylin. *C*, *Ch*, chondrioconts; *Gr*, lipide granules, brown with osmium; *N*, nucleus, sufficiently destained in A and C to show structure.

Reserve lipides which are found in many cells appear, in the cytoplasm, like the microsomes which we have just been discussing but are present in much greater quantity. In endosperm cells of the castor bean for example, at the period immediately preceding the maturation of the seed, there are seen to form abruptly in the cytoplasm, numerous small granules comparable to those which normally exist in every cell and which present the same histochemical characteristics. These finally fill the cytoplasm completely and then fuse into large lipide globules. In the cytoplasm of some filaments of *Saprolegnia*, especially in the extremities of the filaments which will form the zoosporangia, numerous granules are also seen to appear which are comparable to the small microsomes encountered in the other filaments. These fuse later into larger globules which accumulate in the zoospores and serve as reserves.

Fatty degeneration:- Fatty degeneration which is exhibited by many cells at the end of their development, especially in the fungi, is also brought about by the increasingly large production of granules, similar to the microsomes, which run together into large globules. This process seems to correspond to a lipophanerosis *i.e.*, a breaking down of the lipides, of the lipoprotein compounds which comprise the cytoplasm.

Essential oils and resins likewise appear as small globules in the cytoplasm and their very refractive appearance and their histochemical reactions greatly resemble those of the lipide granules, from which they are only with difficulty distinguished.

Other metabolic products:- The cytoplasm of plant cells may contain a great number of other substances arising from cellular metabolism. These substances, however, are not constant like those mentioned above and are determined by certain physiological states of the cell. Some of these products are inclusions in the cytoplasm.

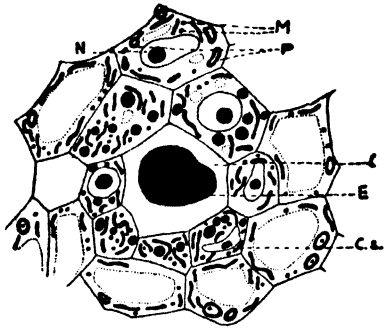


FIG. 142. — *Philodendron*. Secretion canal of an aerial root. *C*, lumen of the canal; *C.s.*, secreting cells; *E*, fatty secretion blackened by osmic acid; *M*, chondriosomes; *N*, nucleus; *P*, plastids. Meves' method with acid fuchsin. (After Miss POPOVICI).

Among them must be mentioned that which is called *floridean starch* or starch of the Rhodophyceae. It differs essentially from ordinary starch by the fact that it is not formed in plastids but appears in the cytoplasm. This starch becomes visible in the cytoplasm as granules of variable dimensions, some extremely small, a fraction of a μ, others of a diameter which may exceed 20-30μ. These variously shaped granules (ovoid, plano-convex, bi-concave, discs etc.) are doubly refractive, if they are large enough, and stain mahogany-brown to violet-red with iodine. The chemical nature of this "floridean starch" is not yet fully determined. It seems however that various substances have been described under this name. Some of them, appearing as rather large granules, correspond to a special variety of starch (VAN TIEGHEM, KYLIN). Others exist in the cytoplasm as a cluster of minute granules capable even of emigrating into the vacuoles. Among these, some seem to be composed of a substance related to the glycogens (ERRERA, COLIN); others are products (alkaloids) still imperfectly known (GUÉGUEN).

In the cytoplasm of the Euglenas are likewise found granules of paramylum characteristic of the Phytoflagellates. These granules appear in the cytoplasm as discs, prisms, rods, stars and so forth, showing alternately dark and light concentric layers like the starch grains. They do not stain with iodine but have been compared to starch. Mention must also be made of para-glycogen,

a substance related to glycogen which is sometimes encountered in bacteria and Phytoflagellates as granules which stain brown with iodine.

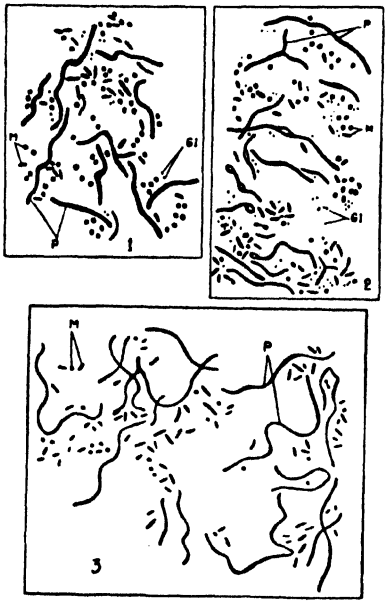


FIG. 143. — *Iris germanica*. Chondriosomes (*M*) and plastids (*P*) in the form of chondrioconts in epidermal cells of the leaf. 1, living material. 2, Meves' method with acid fuchsin. 3, Regaud's method. Lipide granules (*Gl*) very refractive in 1, blackened by osmium in 2, not preserved in 3.

Protein crystalloids are sometimes encountered also in the cytoplasm. In the tubers of white potatoes, for example, a crystalloid of protein is found in the cytoplasm of most of the cells. It is rather large and cubical in shape and is deeply stained by iron haematoxylin. Similar variously-shaped crystalloids (spindle-shaped, cubical etc.) often exist in the plectenchyma of the carpospore of the Agaricaceae and in the mycelium of *Spermophthora gossypii*, as well as in the Mucors, in which they have been described under the name of crystalloids of mucorine (VAN TIEGHEM).

It is suitable also to mention a voluminous spherical inclusion which is present in the cytoplasm of certain special cells of various Rhodophyceae. This is called an *iodine reservoir* (Fr. *ioduque*), is very refractive and occupies the major part of the cell. Its nature is still unknown but it seems to contain iodine which is free or in the state of an unstable compound released when the reservoirs are broken.

Attention has been called in the same algae to cells called *bromine reservoirs* (Fr. *bromuques*), which rather analogously contain bromine. Let us mention furthermore that there exist in the proto-

plasm of the Thiobacteriales, very refractive granules which appear to be sulphur.

Many metabolic products are diffuse in the cytoplasm and cannot be detected, but others can be brought out by microchemical reagents. Among these is glycogen which is so widely distributed in fungi (Fig. 145) and which can be detected by the iodine-potassium

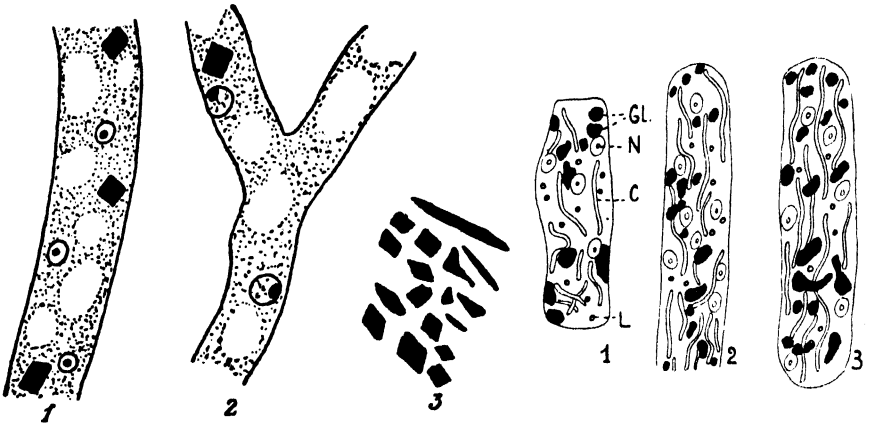


FIG. 144 (left). — *Spermophthora gossypii*. 1, 2, Filaments with crystals of protein. 3, Detail of crystals.

FIG. 145 (right). — *Endomyces Magnusii*. Living oidia and tip of a filament treated with iodine-iodide. Small acacia-brown areas of glycogen (*GL*) are sometimes near the nuclei (*N*) but are not related to the chondriosomes (*C*). *L*, lipide granules.

iodide reagent, giving a mahogany-brown color. This product appears directly in the cytoplasm, generally around the vacuoles, or the nuclei, in small areas which later run together and fill the entire cytoplasm. At times when it accumulates in too great quantity in the cytoplasm, the glycogen may even spread out into the vacuoles, where it often is precipitated as small slightly refractive globules. Lastly there are the amyloids, substances colored blue by iodine which are diffused in the cytoplasm of some bacteria.

Chapter XIX

CYTOPLASMIC ALTERATIONS

It is impossible to discuss fully the vast and, moreover, incompletely known, question of cytoplasmic alterations. We shall consider very briefly: (1) the disturbances which accompany the natural death of cells; (2) the morphological alterations which various physical agents provoke in cells; (3) the reactions shown by the cytoplasm and its morphological constituents under the influence of parasites.

Alterations produced in dying cells:- When living cells of any tissue are examined, even in Ringer's solution, it is always seen that they more or less quickly manifest those signs of alteration which, sooner or later, end in their death. Such alterations seem inevitable. They are explained by the artificiality of the medium in which the cells are placed, by the pressure of the cover glass, by the lack of air, and by the too intense lighting which is, nevertheless, necessary for observation. This is what makes the study of living material so difficult. One manages to retard these alterations by examining leaves or bracts which are protected by their cuticle but are so thin as to be transparent. The cells are altered, however, in the region where the organ has been severed from the plant and the alteration is then transmitted, more or less rapidly, to all the cells. The complicating factor is that one can not know exactly when the alteration of the cell begins. By the movements of the cytoplasm, it is possible to determine whether a cell is living. As long as cytoplasmic movements continue, the cell is living and does not present any important alteration. Nevertheless, cytoplasmic movements do not prove that the cell has not already manifested alterations, for, in general, all wounding causes marked acceleration of the cytoplasmic currents and there are cells in which these movements are to be induced only by lesion. The alteration of the cytoplasm is manifested by a thickening of the chondriosomes and of the plastids, soon afterward accompanied by their transformation into vesicles and later, by interruption of cytoplasmic circulation and its replacement by Brownian movement.

It is difficult to know where the first alterations of the cell begin. We possess relatively accurate data as to the moment at which death of the cell occurs. A first sign of death in a cell is shown when the vacuole, stained with neutral red (the only element stained in the living cell), abruptly loses its color and the dye is taken up by the nucleus and cytoplasm. This is a universal phenomenon which seems to be brought about by a modification of the perivacuolar membrane, permitting diffusion of the dye accumulated in the vacuole. This phenomenon may be compared to

that of *nuclear autochromatism* reported in certain cells whose vacuoles contain anthocyanin (P. A. DANGEARD, MOREAU, GUILLIERMOND). These writers observed that at the moment of death of these cells, the vacuoles lose their color and the pigment becomes localized in the cytoplasm and especially in the nucleus. This phenomenon is observed particularly in the final stages of plasmolysis.

BECQUEREL advocates the use of a mixture of neutral red and methylene blue. Neutral red stains the vacuoles of living cells and the less penetrating methylene blue colors only the cytoplasm of dead cells. The examination of the cells with the ultramicroscope, as has been seen, makes it easily possible to determine the moment when the cell dies.

As soon as death occurs, *i.e.*, the coagulation of the cytoplasm, one witnesses a series of phenomena, designated as *autolysis*, which consist of an autodigestion of the protoplasm under the action of intracellular proteolytic enzymes. The enzymes, whose action is no longer inhibited, induce modifications in the cell, characteristic of degeneration, *i.e.*, cellular necrobiosis. This consists essentially of an autolysis, *i.e.*, of a digestion, starting in the interior of the cell itself and instigated by the enzymes. These enzymes, although present during life, do not act on living material because of some still unknown mechanism.

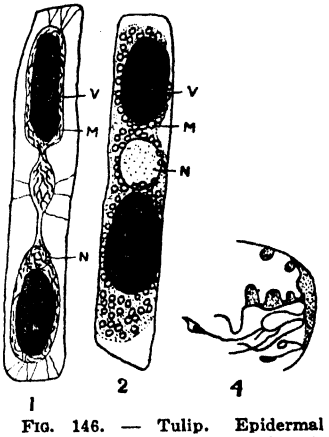


FIG. 146. — Tulip. Epidermal cells of red and yellow petals. 1, living and plasmolyzed. 2, dead, plastids vesiculated. 4, myelin figures formed by the cytoplasm during plasmolysis. *M*, chromoplasts; *N*, nucleus; *V*, vacuole containing anthocyanin.

The modifications generally produced in the cell take the form of more and more marked vesiculation of the chondriosomes and plastids, bringing about the alveolar structure described by BÜTSCHLI. The mitochondrial vesicles, which are often enormous, sometimes finally burst. Their wall then breaks up into an infinity of small refractive granules which are scattered about in the cell.

In its turn, the vacuole ceases to exist when the perivacuolar membrane is destroyed. This leads to a contraction of the cytoplasm which becomes detached from the cellulose wall as if plasmolyzed, and appears as a granular-aveolar coagulum immersed in the liquid of the cell cavity.

The degeneration of the epidermal cells of *Iris* during the fading of the flower, involves curious phenomena associated with the chondriosomes and plastids. The plastids (leucoplasts, chloroplasts and chromoplasts) fill with an infinity of small granules which reduce osmium. These swell and take on the aspect of enormous vesicles. Then the contour of the vesicles gradually loses its distinctness and finally becomes invisible. There remains of the vesicle, therefore, only a mass of lipide globules which have

a tendency to fuse into large globules, while the substratum of the plastid is reduced to a great number of small granules which soon disappear. At the same time, the chondriosomes swell and become vesicular, as do the lipide granules. Eventually there persist in the cytoplasm only large lipide globules, produced by the disorganization of the plastids.

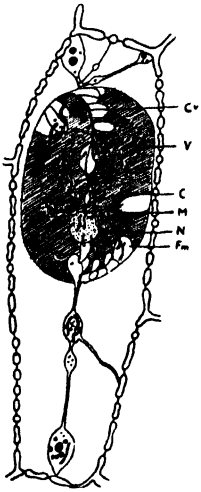


FIG. 147. — *Iris germanica*. Epidermal cell of a leaf plasmolyzed in a 5% NaCl solution with neutral red added. Cell contents form a large ball in the center of the cell connected to the wall by slender threads which are enlarged here and there. *Cv*, bodies precipitated from the colloidal contents of the vacuole by the dye; *V*, vacuole stained with neutral red; *C*, chondriosomes; *M*, mitochondria; *N*, nucleus; *Fm*, structures resembling myelin figures produced from the cytoplasm.

In the chlorophyll-containing tissue, these globules appear green and in cells enclosing xanthophyll they appear yellow, the pigments having dissolved in the lipides during degeneration. These modifications seem to be due to a breaking down of the lipides in the lipoprotein complex comprising the plastids (lipophanerosis). It is apparently phenomena of this same order, produced in the cytoplasm itself, which bring about the *fatty degeneration* so often found in animal cells and in fungi.

In other flowers (*Gladiolus*, tulip, *Clivia*), only the vesiculation of the plastids and chondriosomes is observed. The vesicles burst and disintegrate into small refractive granules which are scattered in the cytoplasm and do not reduce osmium. There is no fatty degeneration.

Alterations produced by various physical agents:- Radiations of short wave length, ultraviolet rays and X rays, as well as α particles emitted by radioactive bodies, destroy living cells, but this destruction, especially by X ray and α particles is not instantaneous. It is, on the contrary, preceded by very complex phenomena concerning which we have, as yet, very little precise knowledge.

The action of ultraviolet rays and X rays on the morphological constituents of the cytoplasm is recorded largely in the work of NADSON and his students ROCHLIN and STERN. These investigations have used as subjects, various yeasts and the epidermal cells of *Allium Cepa*. These workers report in all cases that radiation produced first an excitation of cytoplasmic activity. The currents become more rapid, the cytoplasm forms amoeboid prolongations in the direction of the vacuole, causing it constantly to be deformed. In the second phase, the vacuoles return to their previous shape. At the same time, lipide droplets form in large numbers in the cytoplasm, in the chondriosomes and in the plastids. Finally, plasmolysis occurs and the cells soon die.

Radium and its salts produce similar effects, as shown by NADSON and his followers, as well as by MILOVIDOV. These workers have also studied the action of the radioactive salts on the chondri-

ome. Now this action seems to be very slight. The chondriome, so sensitive to most exterior agents, is only very gradually injured. The lesions consist of a vesiculization of the chondriosomes and of the plastids, accompanied by their fatty degeneration (lipophanerosis). In short, the effect of radium salts is to accelerate the degeneration which would normally be produced much later on.

Plasmolysis leads to interesting modifications of the cell. Myelin figures appear in the cytoplasm (Figs. 146-148) in the form of pediculate buds on the border of the vacuole, which are capable of being detached and of emigrating into the vacuoles. These figures do not seem to be doubly refractive. They may perhaps be attributed to a sort of unmasking of the cytoplasmic lipides. During plasmolysis, there is also often observed a fragmentation of the vacuole into small filamentous or reticulate elements. The chondriosomes and plastids are not modified as long as the cell remains living, but they become vesiculate as soon as the cell dies (GUILLIERMOND, KÜSTER). Let us add that plasmolysis of living but injured cells produces the curious phenomenon described by KÜSTER as *plasmoschism*, in which the permeability of the ectoplasmic membrane is so greatly increased that it no longer inhibits penetration. The perivacuolar membrane, however, retains its semi-permeability and so the vacuole contracts but the irremediably injured cytoplasm is stretched, taking very irregular shapes and breaking apart.

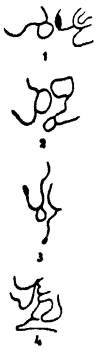


FIG. 148. — *Iris*. Figures like myelin figures in a plasmolyzed epidermal cell.

The study of the death of cells by freezing gave rise to the work of MATRUCHOT and MOLLIARD who thought that freezing brought about plasmolysis because the cold causes dehydration. According to BECQUEREL death by coagulation results from syneresis.

Lastly, the investigations of Miss ROLLEN concerning the action of ethyl chloride, octanol and amyl alcohols on cells has shown that these substances cause a slight plasmolysis of the cell and the cavulation of the chondriosomes.

Alterations produced by parasites:- The processes of alteration of the cytoplasm by parasites are very little known. However, modifications in plastids, in chondriosomes and in vacuoles have been described. BEAUVERIE found a rarefaction of the chloroplasts and chondriosomes in certain parasitized tissues. He explains this by modifications produced by the parasite in the osmotic state of the cells because the parasite secretes enzymes and toxic substances. BEAUVERIE says, however, that these appear only in connection with necrosis, and not when the parasites cause overactivity, as in the case of tumors or in active amylogenesis. In the case in which the alteration takes the form of a diminution in the number of chloroplasts in a cell attacked in the adult state, their dis-

appearance and the phenomena which accompany it, must take place very quickly, and generally escape notice, for here we are dealing with disturbed equilibrium which is, of necessity, temporary. In an attempt to avoid this difficulty and find out what occurs at the moment when the parasite exerts its disorganizing action, BEAUVERIE experimented with various substances which act upon living tissue (notably anisotropic solutions and certain substances such as saponin, which lowers the surface tension, cholesterol and ether which modify the water content of the plastids, etc.). He observed particularly the effects produced on the plastids and chondriosomes. Among his results we find that the chloroplasts may degenerate by spreading out and fusing or, under other conditions, may swell into relatively enormous vesicles which the green pigment covers like a cap. These phenomena may or may not be accompanied by lipophanerosis.

BEAUVERIE tried to show that in certain cases of parasitism the plastids have a tendency to become more fragile (*fragilization*) which is manifested by their greater sensitivity to anisotonic action. This must be a condition preceding their disappearance in the parasitized cell. This diminution of resistance in the plastids in some pathological states brings to mind observations made on blood globules under similar conditions (hemolysis).

BEAUVERIE and other writers have also found a fatty degeneration of the plastids, analogous to that observed in the natural degeneration of cells of some plants (*Iris* discussed above). Vesiculation of the chondriosomes was also observed.

In all cases in which parasitisms has an exciting action on the tissues of the host, this action seems to be manifested by an exaggerated activity of the plastids, if we may judge from the similar results of various investigations on the subject.

Several authors, LJUBIMENKO, DUCOMET, MORQUER, BEAUVERIE, Miss RUTH ALLEN, and DUFRÉNOY, have cited cases in which the parasite not only does not bring about a degeneration of the chloroplasts, but can induce manifestations of super activity, for example, the production of starch more intensely in the parasitized, than in the healthy, portion of the tissue. The research of DUFRÉNOY on a certain number of diseases seems to show that parasites have an exciting action on the tissues of the host which is manifested by an exaggerated plastidial activity. There may be a superabundance of starch which results in a rather considerable increase in leucoplasts. These investigations also show us that fungal parasites exercise an action on the vacuoles of the host which, in cells neighboring on the regions infected, frequently modify their appearance: the large vacuoles break up into numerous minute, filamentous, and reticulate vacuoles, analogous to those observed in embryonic cells. This would seem to indicate an intense secretory activity, facilitated by the increase of surfaces of contact between the cytoplasm and the vacuolar contents. This phase of hyperactivity seems to be followed, in those plants which do not resist infection, by a phase of disintegration of the cyto-

plasmic constituents, during which soluble nitrogenous matter and lipide droplets are set free. In plants which resist infection, the action of the parasite brings about an intense production of tannins and anthocyanin pigments.

Chapter XX

SUMMARY AND CONCLUSIONS

In the account which has just been given, an endeavor has been made to set forth all the facts now known concerning the cytoplasm and the considerable progress which has been made during these last years with regard to this important question. Until 1910, there were only hypothetical and quite fragmentary data on the cytoplasm, although the nucleus had been exclusively studied. At the present time, the subject of the physical constitution of the cytoplasm has made rapid progress and its morphological constitution is today definitively clarified. In concluding this volume, it is well to summarize the knowledge which we possess on the subject at the present time, in order to bring out essential features and, above all, to give a comprehensive view of the morphological constitution of the cytoplasm.

Recent investigations have shown the cytoplasm to be a very complex heterogeneous structure.

Cytoplasm:- It is now proved that the cytoplasm, to which cytologists until 1908 had attributed a special structure, generally reticular or alveolar, appears, on the contrary, as a homogeneous and transparent substance. It is essentially composed of a lipoprotein complex (protein, about 55% and lipides about 15% of dry weight) in pseudosolution in water (about 80-90%) containing mineral salts. Cytoplasm offers characteristics intermediate between liquids and solids. It is fluid, it moves and it exhibits surface tension, *i.e.*, whenever it is extracted from the cell it tends to take the shape of minimum surface, namely, the spherical form. These are properties of liquids. But it also has rigidity, giving it a torsion elasticity which is a property of solids. Its viscosity has been measured and found to vary according to the physiological state of the cell and the physical condition in which the cell is placed. Its viscosity is always superior to that of water but may be very much higher and comparable to that of glycerine or even of oil of vaseline. In dehydrated organs, the cytoplasm may become more or less solid.

Since the celebrated work of MAYER and SCHAEFFER on animal cells (1908), confirmed by that of LAPICQUE, BECQUEREL and GUILLIERMOND on plant cells, it has been recognized that the cytoplasm of living cells always appears optically empty under the ultramicroscope, just as do solutions of nucleoprotein. The cytoplasm behaves like an electronegative hydrogel. Like all gels, alkaline or negative, it becomes cloudy when acids are introduced into it: First luminous streaks, then ultramicroscopic granules are seen to appear, and the cytoplasm is transformed into a network of gran-

ules which render it entirely luminous. It takes on the aspect of a lump of snow and is then coagulated.

Since the work of MAYER and SCHAEFFER, it has been believed that the cytoplasm is in the state of a *fluid hydrogel.* Nevertheless, the cytoplasm differs essentially from gels by the fact that it is not miscible with water. Because of this special characteristic, BOTTAZZI had given to the colloidal system existing only in plant cells, the name *gliode.* It seems to us at the present time, in consequence of the research of BUNGENBERG DE JONG, that it can be likened to a coacervate system.

Excluding the bacteria and Cyanophyceae (inferior organisms with a very primitive structure), investigations of plant cells since 1910 have proved that each cell, and this is also true for animals, can be considered as containing permanently in suspension in its homogeneous and transparent cytoplasm, small elements presenting well defined morphological and histochemical characteristics, namely, the *chondriosomes.* These appear as cellular entities. The entire group of them in a cell constitutes the *chondriome.* Together with these elements, there are found in the cytoplasm of green plants other cellular entities, presenting during functional inactivity the same forms and the same histochemical characteristics as the chondriosomes, therefore closely related to them, and indistinguishable from them except for an ability to manufacture chlorophyll and starch. These are the *plastids* which can be considered as a variety of chondriosome peculiar to chlorophyll-containing plants and existing because of the photosynthetic processes which characterize green plants.

Finally, every cell, even those of bacteria and the Cyanophyceae, contains a *vacuolar system* or *vacuome,* and in addition a more or less large quantity of *lipide granules* ("microsomes" of other authors).

The chondriome:- The chondriome is composed of elements showing by their shapes as well as by their dimensions, a great resemblance to bacteria. These are elements in the form of granules (mitochondria), of short rods, or of filaments which may be grouped or branched (chondrioconts, 0.5μ-1μ thick). They are capable of dividing and of passing from one to the other of these forms, either by elongations of the mitochondria or by fragmentation of the chondrioconts. The mitochondrial form is generally the one which characterizes sexual cells and the early stages of embryonic cells. The chondriocontal form is generally the one which is the most wide-spread throughout differentiated cells. It seems, as MEVES states, that the chondriosomes must be considered as permanent entities of the cell, incapable of arising *de novo* and capable of being transmitted from cell to cell by division. This continuity of chondriosomes is rendered very probable by the fact that during sporulation of the fungi (Ascomycetes, Saprolegniaceae, *Allomyces,* etc.) these elements show division figures and are distributed among the spores. This continuity is, however, im-

possible to demonstrate but is proved without a doubt in the case of the plastids which appear to be only a special category of chondriosomes, and furnish an important though indirect argument in favor of this opinion.

The chondriosomes are visible in living material in which they appear as slightly refractive elements whose forms are exactly like those obtained in fixed preparations. They are more difficult to

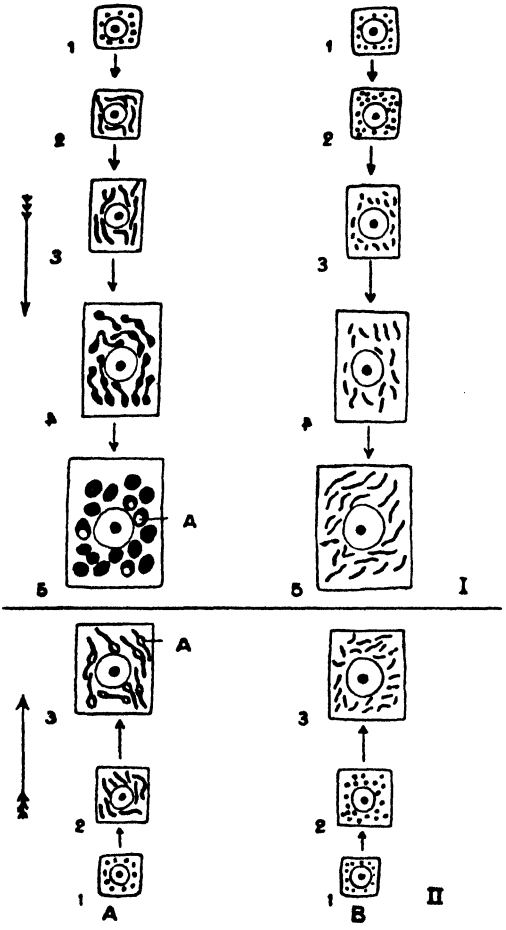


FIG. 149. — Diagram of the development of the chondriome in Phanerogams. A, plastids. B, genuine chondriosomes. I, stem and leaves. II, root. *A*, starch.

distinguish under the ultramicroscope, for they appear in black with a faintly luminous contour which seems to indicate that, like the cytoplasm, they are in the state of a hydrogel or of a coacervate. The chondriosomes are slowly displaced by the cytoplasmic currents and constantly change shape (Figs. 70, 150), which proves that they are composed of a semi-fluid and very plastic substance. They have a specific weight rather close to that of the cytoplasm

and are not displaced by centrifuging. They behave like extremely fragile elements, changing very readily under various physico-chemical influences (disturbances in osmotic equilibrium, pressure on the cover glass). When altered, they swell and then become transformed into large vesicles (*cavulation*).

Living chondriosomes can be stained with Janus green, Dahlia violet, methyl violet and other dyes. Staining of the chondriosomes is vital only if these dyes are used at very low concentrations. Otherwise it is sublethal and after a certain time causes the

FIG. 150. — *Allium Cepa*. Various forms taken by chondriosomes (*M*) and leucoplasts (*P*) in living epidermal cells of the bulb. *Gg*, lipide granules.

death of the cells.

From a chemical point of view, the chondriosomes have a lipoprotein constitution and seem to be composed of a protein and phosphoaminolipide complex which is much richer in lipides than is the cytoplasm. The chemical behavior of the chondriosomes is absolutely different from that of chromatin and they do not show the Feulgen reaction. Their lipide content gives them a series of very definite histochemical characteristics. They are profoundly altered by fixatives containing alcohol or acetic acid; they are preserved and show a predilection for staining only with special methods called mitochondrial methods; they are stained because of the lipides in their substratum.

There has been attributed to chondriosomes in animal cells a preponderant and direct rôle in the elaboration of metabolic products: fats, granules resulting from all sorts of secretion, pigments. According to this conception the chondriosomes have a rôle rather analogous to that of plastids in chlorophyll-containing plants. This conception seemed, for a while, all the better established since the later research in plant cytology shows that the plastids of the phanerogams have exactly the same morphological and histochemical characteristics as the chondriosomes. Further research did not, however, confirm this rôle, at least, in most cases, and if it exists, it is very limited. The rôle of the chondriosomes is still unknown. Nevertheless the fact that they are closely related to the plastids of chlorophyll-containing plants permits us to imagine that the chondriosomes have a very important function in cellular metabolism.

The plastids:- The cells of chlorophyll-containing plants are distinguished from the cells of animals and fungi by the presence of a second category of organelles, the *plastids*. It is definitively proved that these are organelles which form only by division of pre-existing plastids and which maintain their individuality in the course of development. In those higher plants in which chlorophyll is not continuously elaborated, the plastids which are functionally inactive have the same forms and the same histochemical characteristics as the chondriosomes, among which they can not be identified in embryonic cells. Forming with them a chondriome of homogeneous appearance, the plastids are distinguishable only by their power, during cellular differentiation, of elaborating or accumulating chlorophyll, carotinoid pigments and starch, and by the fact that the products which they engender modify their shapes. This modification is only transitory in the case of starch. The starch grain formed within the plastid is hydrolyzed in the interior of the plastid which then recovers its inactive shape until such time as there is a new elaboration of starch. Depending upon the case in question, the plastid is transitory or permanent. During the production of chlorophyll, the plastid, in filling up with pigment, hypertrophies and appears as a large spherical body. It may remain indefinitely in this state or, in some cases, it may lose its chlorophyll and appear again as a minute chondriosome. Plants exist, many algae and bryophytes for example, in which chlorophyll is permanently secreted and in which all the cells, at the same time, contain both large highly differentiated chloroplasts and small chondriosomes, both transmitted by division from cell to cell. The existence of such groups furnishes proof that, in the higher plants, the chondriosomes capable of elaborating starch, or of accumulating chlorophyll, do not have genetic relationships with those lacking this function. There exists in embryonic cells of *Selaginella* a single chloroplast, shaped like a chondriocont and differing from the other chondriosomes by its slightly larger size. This, together with *Anthoceros* which has a single but

much smaller chloroplast, provides an intermediate step between algae which have only a single, permanent, very differentiated,

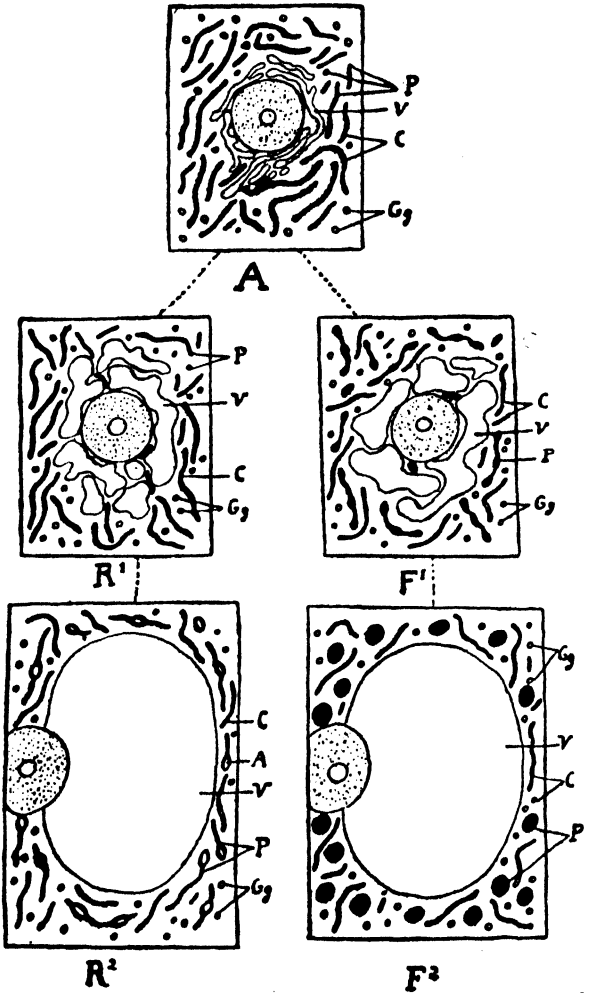


FIG. 151. — Diagrammatic representation of the structure of phanerogam cells during development, based upon the author's investigations. A, embryonic cells; colorless plastids (*P*) shaped like filaments, rods, and granules, indistinguishable from the genuine chondriosomes (*C*); chondriosome-shaped vacuoles (*V*) looking like canaliculi of Holmgren; *Gg*, lipide granules (microsomes of other authors) which do not stain with mitochondrial techniques. R_1, cell at the beginning of differentiation in root; vacuoles enlarging and coalescing; plastids and chondriosomes unchanged. R_2, mature cell of root; a single large vacuole: plastids show vesicles with starch (*A*) which mitochondrial techniques do not stain. F_1, cell of leaf primordium; vacuoles enlarging and coalescing; plastids form swellings which increase in size, separate and become chloroplasts. F_2, mature cell of leaf; a single vacuole; large chloroplasts easily distinguishable from the chondriosomes.

chloroplast, on the one hand and the phanerogams, on the other, in whose embryonic cells no distinction whatever can be established between the plastids and ordinary chondriosomes.

As the plastids have, furthermore, histochemical and histophysical characteristics entirely similar to those of the chondriosomes (viscosity, manner of alteration, refraction, behavior in regard to fixatives and stains), it is legitimate to put them in the same category. The plastids may be considered as a supplementary category of chondriosomes connected with the photosynthetic function which characterizes green plants. Hence we are led to think that the ordinary chondriosomes and the plastids, by virtue of their finely divided state in the cytoplasm, are the seat of important surface phenomena and that they have a similar general function, of which that shown by the plastids is one manifestation.

Vacuolar system or vacuome:- The vacuolar system, or vacuome, is represented in embryonic cells of most plants by numerous minute inclusions of semi-fluid consistency composed of a very concentrated colloidal solution (in the state of a hydrogel or coacervate). In their forms (granules, isolated or assembled in chains, undulating filaments often anastomosing into a network), they sometimes greatly resemble the chondriosomes. These inclusions are occasionally visible in living material because they are more refractive than the cytoplasm, or because they contain anthocyanin pigments which give them a natural color. They are most difficult to distinguish under the ultramicroscope, for here they look like chondriosomes. They can be easily brought out with vital stains (neutral red, cresyl blue, Nile blue), for which they have a strong predilection. They are stained homogeneously and deeply by these dyes without precipitation of the enclosed colloids. They have, however, less affinity for chondriosomal stains (Janus green, Dahlia violet and methyl violet) which, nevertheless, also stain them when they contain substances capable of retaining these dyes, in particular phenol compounds, and when the dyes used are of certain concentrations. In the course of cellular differentiation, these elements swell because they contain colloids in pseudosolution whose capacity for taking in water is much greater than that of the cytoplasm. Thus they are transformed into small, spherical, increasingly fluid vacuoles (vacuoles in the classical sense). There has taken place, therefore, a transformation of the very condensed colloidal substance, of which they seem to have been formed, into a very dilute solution. The vacuoles may later fuse so that, in the mature cells, there is formed a single enormous vacuole. As they become liquid, the vacuoles cease to stain homogeneously and deeply with vital stains, and their colloids are precipitated as deeply colored corpuscles showing Brownian movement in the vacuolar sap. This last remains colorless, or takes a diffuse tint. Sometimes the dyes do not cause a precipitation and the vacuolar sap stains only diffusely. In certain cases, as they swell and fuse together, the colloidal solutions of the young chondriome-shaped vacuoles continue to be very concentrated, and the large vacuole of the mature cells continues to have a colloidal content in the state of a hydrogel or of an almost solid gel which, by syneresis,

may, under some conditions, separate from the vacuolar liquid (*vacuolar contraction*).

The large vacuole of mature cells is capable, under certain influences, of losing its water and of fragmenting into minute, semi-fluid, chondriosome-shaped elements. The various aspects of the vacuolar system are, therefore, reversible and seem to depend upon the water content of the cytoplasm. Water may move into the cytoplasm and out of the vacuoles and the reverse action may take place. The vacuoles themselves, during dehydration of the seed, are capable of losing water to the point of being transformed into solid bodies (*aleurone grains*) which later, at germination, again become vacuoles after taking in water.

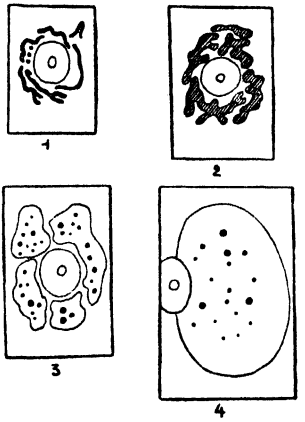


FIG. 152. — Diagrammatic representation of the vacuolar system in phanerogam cells vitally stained with neutral red. 1, embryonic cell; small, semi-fluid, chondriosome-shaped vacuoles composed of a very condensed colloidal substance, homogeneously and deeply stained. 2, differentiating cell; vacuoles enlarged by absorption of water and united in a network. 3, later stage; small vacuoles unite to form a few large, unstained, liquid vacuoles containing a dilute colloidal solution; the dye causes flocculation of the colloids as deeply stained precipitates showing Brownian movement. 4, mature cell; fusion of vacuoles to form a single large one with deeply stained precipitates.

Although in their semi-fluid state the vacuoles may very much resemble the chondriosomes and the plastids, they are always distinguishable from these elements by their histochemical behavior, notably by their instantaneous staining with vital dyes, such as neutral red and cresyl blue, which stain neither the chondriosomes nor the plastids. They are to be distinguished from these elements also by the fact that the staining is essentially vital and ceases as soon as death of the cells occurs, whereupon the protoplasm is stained. This is very different from the sub-lethal staining of the chondriome which almost never occurs except in dying cells and persists after the death of the cells.

Furthermore, the histochemical behavior of the vacuoles is very variable and even the chondriosome-shaped vacuoles differ essentially from the chondriosomes, by the fact that the former have no defined characteristics. In general, they do not stain either by mitochondrial techniques or by other methods of fixation but in all well differentiated preparations they appear as colorless canaliculi. In the case in which they are stained by mitochondrial techniques, they then appear as small vacuoles in which the colloidal content has been precipitated and stained. This does not permit them to be confused with the chondriosomes. Aleurone grains, which are dehydrated vacuoles, always stain with mitochondrial techniques and without their contents being precipitated. In some lower plants, fungi for example, the chondriosome-shaped vacuoles contain a substance called metachromatin, capable of being precipitated with alcohol, but which does not stain with mitochondrial

technique. It has no marked affinity for ferric lake, but can be stained by other processes (with cresyl blue, haematein and other dyes), which give the vacuoles a typical reddish color characteristic of metachromatin.

It is possible, on the other hand, to stain both the chondriome and the vacuolar system at the same time in living material, by a mixture of Janus green and neutral red, and then it can be observed that the two systems are always coexistent and have no genetical relationship whatever. There is, therefore, between the vacuoles which are shaped like chondriosomes and the chondriosomes themselves, only a similarity of form, which doubtless represents a somewhat closely related physical state of these two categories of elements. In some lower algae, the vacuoles do not undergo hydration and during the entire development of the cells persist as numerous semi-fluid inclusions, scattered about in the cytoplasm, analogous to those which characterize the embryonic state of cells of higher plants. The vacuoles of animal cells seem to be of this type. In other algae, on the contrary, the vacuoles are always large liquid vacuoles.

The vacuoles, in all stages of their development, almost always contain colloidal substances dispersed in their sap and vital staining of the vacuoles is connected with the presence of these substances. These substances, however, vary in nature according to the type of cell and correspond to the products secreted by the cell. There is no substance characteristic of vacuoles as there is of chondriosomes. Often there are encountered in the same cell two categories of vacuoles, always independent of each other, made distinct by their colloidal content. In other cases there are encountered, side by side in a single cell, vacuoles with colloidal contents and others seeming not to contain any colloidal substance whatever. These latter seem to have no predilection for vital dyes, yet seem to be derived from the former by a phenomenon of syneresis or of coacervation.

Although almost always represented in plant cells, and capable of fragmenting, the vacuoles seem to arise *de novo*. It may be supposed that their formation is, in general, connected with secretory phenomena of the cell. Each colloidal granule secreted by the cytoplasm, possessing a capacity for taking in water which is greater than that of the cytoplasm, seems capable of engendering a vacuole. There are, as well, many other inclusions but they are purely transitory and dependent on the physiological state of the cell. These are products arising from cellular metabolism.

Thus the cell can be made to fit into a general plan which applies to every cell, animal as well as plant, with the exception of the Cyanophyceae and the bacteria. These facts bring out strikingly the extreme complexity of the morphological constitution of the cytoplasm which appears to us as a colloidal system in which the chondriosomes, the plastids and the vacuoles constitute so many distinct phases. The cytoplasm is, therefore, a colloidal system of a very heterogeneous structure.

Unfortunately, the very precise knowledge which we possess at the present time on the constitution of the cytoplasm consists almost exclusively of morphological facts. We still know only very little about the rôle of the different elements which compose this structure. We know that the plastids play an important part in the phenomena of photosynthesis and in amylogenesis and that the vacuoles play a part in the osmotic phenomena of the cell. Our knowledge ceases here. But, as CLAUDE BERNARD says, "Anatomy would have no reason for being if it did not have a physiological base."

It is, however, very important to know and to understand this morphological structure, for, just as WURMSER wrote, "It is certain that a chemical system composed of a number of bodies will develop differently according to whether these parts are all mingled and thus destined to act mutually or whether, on the contrary, they are distributed as independent groups." Therefore the entire problem will consist, from now on, in seeking the relationship between morphological structure and the physiological activity of the cell. The realization of this problem must be hoped for in the progress of physical chemistry. "From now on", as FRIEDL WEBER says, "the cytologist must become both physicist and chemist". We are constrained, however, to add that the cytologist must at the same time remain a morphologist.

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